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54 Peptides corresponding to antigenic and immunogenic determinants of major neutralizing proteins of rotaviruses.

57 Synthetic peptides corresponding to antigenic determinants of three major neutralizing proteins of bovine rotavirus are disclosed. These peptides are useful in stimulating immunity and interfering with viral infectivity and can be

used as vaccines and in other ways for treatment, prevention or diagnosis of rotavirus infections of birds and animals including man.

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AMINO ACID SEQUENCE OF ROTAVIRUS GP7 GLYCOPROTEIN (The 194 polypeptide is shown in the box)

The present invention relates to peptide fragments of the major outer capsid neutralizing glycoprotein (virus protein 7 or VP7), an inner or nucleocapsid protein (virus protein 6 or VP6), and an
5 outer shell protein (virus protein 3 or VP3) of rotaviruses and their use as vaccines in birds and mammals including man. The invention further relates to the production of relevant peptide fragments of rotavirus, to the attachment of peptides to carriers and to their use as
10 vaccines. An added feature of the VP3 peptide is its ability to compete with rotavirus for trypsin, thus interfering with infectivity of the virus. Therefore, the VP3 peptide may also have therapeutic use.

BRIEF DESCRIPTION OF THE DRAWINGS

15 **FIGURE 1** shows the amino acid sequence of rotavirus VP7 glycoprotein the 14K polypeptide is shown in the box.

FIGURE 2 shows the nucleotide sequence of cloned copy of SA-11 gene 6. The (+) sense strand (corresponding
20 to the mRNA) is shown. The predicted amino acid sequence of the protein product is shown and the termination sites are underlined.

FIGURE 3 shows the amino acid sequence of rotavirus VP3 protein. The C486 (bovine) VP3 amino acid
25 sequence was generated in the laboratory. The SA-11 (simian) VP3 amino acid sequence was from prior art.

FIGURE 4 shows the isolation of a bovine rotavirus (BRV) 14k peptide fragment. Lanes A and B illustrate the digestion pattern of the BRV glycoprotein
30 produced with 65 ug of papain/cm of gel and 130 ug of papain/cm of gel, respectively. Lane C illustrates the purified 14K peptide fragment. Lanes represent immunoblot-ELISA reactions with monoclonal antibodies from hybridoma 11D12-6. Lane D illustrates the purified 14k
35 peptide fragment in a silver stained polyacrylamide gel.

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FIGURE 5 shows the antibody titers to various preparations of the 14k polypeptide at three different times during the immunization schedule. The upper panel shows total antibody titers as determined by ELISA using double-shelled rotavirus as the antigen. The lower panel shows neutralizing antibody titers as determined by plaque reduction assays. Group A, 14k unconjugated poly-peptide; Group B, 14k BSA-conjugated polypeptide; Group C, purified VP7; Group D, infectious bovine rotavirus (BRV); Group E₁, animals given infectious BRV at 61 days; Group E₂, animals given adjuvant. The immunization protocol is described in more detail in Table 2.

FIGURE 6 shows the immuno-blot ELISA reaction of mouse sera with the C486 bovine rotavirus (BRV) protein profile. Lane P, prebleed; Lane A, group given 14k unconjugated peptide; Lane B, 14k conjugated peptide; Lane C, group given BRV VP7 (38.2k glycoprotein); Lane D, group given infectious virus; Lane E and E₂, groups given saline and Freund's complete adjuvant respectively. The immunization schedule for each group is described in more detail in Table 2.

FIGURE 7 shows the cleavage patterns of VP7 by Staph aureus V8 protease, papain, chymotrypsin and cyanogen bromide to locate 14k.

FIGURE 8 shows the ability of synthetic peptides of VP7 to block virus attachment to cells.

FIGURE 9 shows the ELISA antibody titers to synthetic peptides.

FIGURE 10 shows the virus neutralizing antibody titres in mice induced by synthetic peptides to VP7.

FIGURE 11 shows the Immunoprecipitation of [³⁵S] methionine-labelled, bovine rotavirus-infected cell lysate by monoclonal 1D7 (lane B); 1B4 (lane C); 1B9 (lane D) and 1D10 (lane E). Lane A; is the reaction with rabbit hyperimmune anti-bovine rotavirus. The position of

the molecular weight markers are demonstrated on the left hand side.

FIGURE 12 shows the reaction of monoclonal antibodies and monospecific polyclonal antisera with bovine rotavirus polypeptides transferred to nitrocellulose. Lane A, monoclonal antibody 1D7; lane B, 1B4; lane C, 1B9; lane D, 1D10 and Lane E, anti-nucleocapsid monospecific antisera. The molecular weights of the reaction proteins are given on the left hand side.

FIGURE 13 shows the immune-blot ELISA reaction of bovine, simian, pig and human rotavirus VP6 nucleocapsid proteins with monoclonal antibody 1B4. Lane A, bovine isolate C486; Lane B, porcine isolate OSU; Lane C, bovine isolate NCDV; Lane D, human isolate Wa; Lane E, bovine isolate UK; Lane F, human isolate ST4; Lane G, simian isolate SA-11. Human isolates Wa and ST4 belong to subgroup 2, while all other isolates belong to sub-group 1. Molecular weights are indicated on the right hand side and ascites control is designated.

FIGURE 14 shows the reaction of bovine rotavirus VP6 nucleocapsid protein digests with monoclonal antibodies 1D7, 1B4, 1B9 and 1D10 and with monospecific antiserum. The enzyme and quantity used per digest are indicated on the figure.

FIGURE 15 shows partial carboxypeptidase digest and Cyanogen bromide chemical cleavage of the VP6 nucleocapsid protein and immunoblot ELISA reaction with monoclonal antibody 1B9. Lanes A-I represent the autoradiogram of the digests; Lanes A'-I' represent the corresponding immunoblot ELISA reaction using monoclonal antibody 1B9. Lanes A, A' 100 ug carboxypeptidase; B, B', 50 ug carboxypeptidase; C, C', 25 ug carboxypeptidase; D, D', 2.5 ug carboxypeptidase; E, E', .25 ug carboxypeptidase; F, F', undigested nucleocapsid; G, G',

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2 h digestion with 50 ug cyanogen bromide; H, H', 1 h digestion with 50 ug cyanogen bromide; I, I', 30 min digestion with 50 ug cyanogen bromide. The positions of molecular weight standards are indicated on the left hand side of the figure.

FIGURE 16 shows the reaction of antiserum to the synthetic peptide 232-256 of VP3 with the rotavirus protein profile. The upper panel (Lane A-H) represents the autoradiograph and the lower panel (Lanes A'-H') represents the immunoblot-ELISA reaction of the protein profile shown in the upper panel with anti-synthetic peptide serum. Lanes A-D represent protein profiles generated in the presence of Laemmli sample buffer containing BME and boiled, and Lanes E-H represent protein profiles generated in the presence of Laemmli sample buffer without BME but boiled. Lanes A, A' and E and E' represent a mixture of 100 ug of synthetic peptide, 2.0 ug double-shelled rotavirus, and .96 ug trypsin. Lanes B and B' and F and F' represent a mixture of 25 ug of synthetic peptide, 2.0 ug of double-shelled rotavirus and .96 ug trypsin. Lanes C and C'; D and D' and G and G' represent 2.0 ug of double-shelled rotavirus and .96 ug trypsin. Lane H and H' represent double-shelled virus. The numbers indicate position of various proteins referred to in the text. Molecular weight markers are indicated on the right hand side.

FIGURE 17 shows the reactivity of synthetic peptide with the VP3 nucleocapsid protein of bovine rotavirus. Lane A represents the protein profile after reacting 2.0 ug of double-shelled virus with 25 ug of synthetic peptide, Lane B represents the same profile as in A after treatment with .96 ug of trypsin. Lane C represents the protein profile after reacting 2.0 ug of double-shelled virus with 100 ug of synthetic peptide and Lane D represents the same profile as in C after treatment

with 0.96 ug of trypsin. Lane E represents the protein profile of 2.0 ug of virus treated with 0.96 ug of trypsin and Lane F represents the protein profile of 2.0 ug of untreated double-shelled virus. The position of molecular weight standards are indicated on the right hand side. The brackets ([]) in Lane C indicate ladders formed in the 45K, 90K and 135K region.

FIGURE 18 shows the reactivity of antisera against the 232-256 VP3 synthetic peptide with the ladder complex on VP6 nucleocapsid monomers and dimers. Lane A represents the virus protein profile. Lane B represents the virus protein profile after complexing with 100 ug of the synthetic peptide and then treatment with .96 ug of trypsin; Lane B' represents the virus profile in Lane B electroblotted and reacted with anti-synthetic peptide antibodies. Lane C represents the virus protein profile after complexing with 100 ug of the synthetic peptide; Lane C' represents the virus profile in Lane C electroblotted and reacted with anti-synthetic peptide antibodies. The right hand side illustrated the location of molecular weight markers.

FIGURE 19 shows the investigation of sample buffer conditions necessary to maintain the 232-256 VP3 synthetic peptide-VP6 complex. Two micrograms of radiolabelled double-shelled rotavirus was reacted with 100 ug synthetic peptide for 30 min at 37°C. Prior to electrophoresis sample was aliquoted and treated with urea sample buffer, Lane A'; Laemmli sample buffer without BME, Lane B; and Laemmli sample buffer containing BME and boiled, Lane C. The arrowheads indicate the position of the 45K, 90K, 135 ladders. The position of the molecular weight standards are located on the right hand side.

FIGURE 20 shows the trypsin cleavage of the synthetic peptide. Lanes B, D and F represent trypsin at 19.2 ug, 9.6 ug and .96 ug respectively. Lane A, C and E

represent the reaction of 100 ug of synthetic peptide with 19.2 ug, 9.6 ug and .96 ug of trypsin, respectively. Lane G represents 100 ug of the peptide with the arrows indicating the position of the monomer and dimer. Lane H indicates the position of molecular weight markers.

FIGURE 21 shows the competition of 232-256 VP3 synthetic peptide with the intact 84,000 VP3 for trypsin. Lane A represents the protein profile of double-shelled rotavirus. Lane C-F represents the viral protein profile after incubation of virus for 30 min at 37°C with .0097 ug of trypsin and increasing amounts of synthetic peptide. Lane B represents no synthetic peptide, Lane C has 25 ug; Lane D, 50 ug; Lane E, 75 ug; and Lane F, 200 ug of synthetic peptide. The positioned molecular weight markers are indicated on the left hand side. The arrowheads at Lane B denote the position of the doublet observed at 60,000 and the arrowhead at Lane F denotes the position of the 84,000 protein.

FIGURE 22 shows the effect of increasing amounts of synthetic peptide on the infectivity of rotavirus. Well A represents MA-104 cell control, well B represents the virus control approximately 100 PFU. Wells C-H represent duplicate samples of 100 PFU adsorbed to MA-104 cell monolayers in the presence of 100 ug, 200 ug and 300 ug of synthetic peptide, respectively.

BACKGROUND OF THE INVENTION

Rotaviruses are important causes of gastrointestinal disorders and diarrhea in a wide variety of avian and animal species including man. The rotavirus genome consists of eleven segments of double-stranded RNA. These eleven genes encode the production of at least six structural proteins of the virus. In complete virus particles, these six proteins occur in a double-shelled arrangement. There are three inner shell proteins designated virus protein (VP) 1, 2 and 6. There are three

outer shell proteins, two of which are designated VP3 and VP7. The third outer capsid protein, which is encoded by genomic segment 10 or 11, has not yet been assigned a number.

The molecular weights of these proteins are shown in Table 1.

Table 1. Gene Assignment and Molecular Weight
of the Major Rotavirus Structural Proteins

Genomic Segment	Protein Designation	Molecular Weight ^a	Location ^b
1	VP1	110K	inner
2	VP2	92K	inner
4	VP3	84K	outer
6	VP6	45K	inner
7			
8 triplet	VP7	41K	outer
9			
10 or 11	ND	20K	outer

9.

- 5 a In different rotaviruses, the absolute order of the genomic segments does not always correspond to the same genes. For example, the electrophoretic order of segments 7, 8 and 9 changes among rotaviruses from different animal species. This is referred to as inversion or "flip-flopping" of genome segments. The gene triplet formed by segments 7, 8 and 9 codes for three polypeptides, the neutralization-specific major outer capsid glycoprotein identified as virus protein (VP) 7, and two nonstructural proteins which are not shown in the table. In rotavirus strains SA-11, W and Wa, gene 9 codes for VP7. In contrast, in rotavirus strain DS-1 and U.K. bovine rotavirus, gene 8 codes for VP7. There are discrepancies in the literature about the exact molecular weight of VP7, as well as of other rotavirus proteins. Several researchers have suggested that this is in part due to the many variations in methods used to: 1) separate the individual polypeptides, 2) prepare virus samples for electrophoresis, 3) detect polypeptides in polyacrylamide gels and 4) detect various post-translational modifications of primary gene products. In addition, especially for bovine and human rotavirus, there are variations in the mobility of proteins derived from different isolates originating from the same species. The molecular weights shown in Table 1 are those reported by Sabara et al (Journal of Virology, Vol 53:58-66, 1985).
- 10 b Designates location of the structural protein in the inner or outer capsid of complete rotavirus particles.

Peptide subunits of VP7, VP6 and VP3 are the topic of this invention.

25 a) Virus Protein 7 (VP7)

The major outer shell polypeptide, VP7, is a glycoprotein with an approximate molecular weight of 38,200 (38.2 K) in its unreduced form and 41,900 (41.9 K) in its reduced form. It has been shown to be the major antigen responsible for inducing neutralizing antibodies to the virus. This glycoprotein is also responsible for virus attachment to cells.

Different serotypes of rotavirus occur and are defined by the neutralizing activity stimulated by VP7. To date, seven serotypes have been identified; four of

these (serotypes 1 to 4) are found in humans and five (serotypes 3 to 7) are found in animals. The importance of these serotypic differences is unclear because recent studies showed that in both animals and man, cross-protection among strains belonging to different serotypes may occur. This cross-protection may occur because there are common antigenic determinants of VP7 which are independent of serotype. Alternatively, the specific amino acid sequences within VP7 (epitopes) responsible for serotype specificity may induce some cross-reactive antibody that is responsible for cross-protection.

Having a molecular weight of 38.2/41.9 K, VP7 is made up of approximately 325 amino acids (Figure 1). The sequence of amino acids comprising VP7 of several different rotavirus isolates has been determined and indicates that the degree of amino acid homology ranges from 75 to 86%. Comparison of the sequences of these VP7's reveal several regions in which the amino acid sequence varies. However, prior to this disclosure, none of these fragments, nor any other similar natural or synthetic material has been used to demonstrate in vivo protective effects.

Epitope mapping of VP7 using neutralizing monoclonal antibodies localized a neutralizing-absorption domain to a component peptide with an approximate molecular weight of 14,000 (14 K). When purified, this 14 K peptide stimulated the formation of neutralizing antibodies in mice. The secondary structure of this peptide, as determined by disulphide bridges, is necessary for maintaining antigenicity. Within this 14 K peptide, four epitopes were identified which possessed some of the biological activity of VP 7 and of complete rotavirus particles.

b) Virus Protein 6 (VP6)

The 45K molecular weight nucleocapsid protein,

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although not an integral component of the outer capsid, is also an important antigen. It has been identified as the subgroup antigen by using several techniques including complement fixation, ELISA, immuno-adherence agglutination assay and specific monoclonal antibodies. The 45K nucleocapsid is also described as the common rotavirus group antigen since some monoclonal antibodies react with all rotaviruses and polyclonal serum raised against a single rotavirus type can detect most other rotavirus strains. Aside from its antigenic properties, the nucleocapsid protein is very immunogenic and several investigators have found that polyclonal serum raised to this protein has neutralizing ability.

Within bovine rotavirus, VP6 appears to exist in trimeric units in both the virus particle and in infected cells, with the intersubunit linkage consisting of noncovalent interactions. These trimeric units complex further by virtue of disulfide bridges into larger units which likely represent the ring-like structures observed by several investigators using electron microscopy. By employing different sample buffers, these nucleocapsid complexes can be visualized on polyacrylamide gels.

Four VP6-specific monoclonal antibodies with low neutralizing ability were produced. They reacted with the monomeric and trimeric units indicating that a site responsible for neutralization was exposed. Taking into account the configuration of the nucleocapsid in the virus particle, such a site can potentially be expressed at least eighteen times, in a tandem fashion, per ring-like structure. Further analysis of radiolabelled virus revealed that a high percentage (80%) of the infectious virus particles were partially double-shelled to the extent that monoclonal antibodies could recognize the VP6 and immunoprecipitate the virus particles. However, only a small percentage of these antibody-bound infectious

virions could be neutralized by the antibody. These results may help to explain the high immunogenicity but low neutralizing ability of VP6.

5 The VP6-specific monoclonal antibodies also cross-reacted with simian (SA-11), pig (OSU), bovine (UK and NCDV), rhesus (RRV), and human (Wa and ST4) rotavirus isolates. Since these viruses belong to different sub-groups, it is likely that the monoclonal antibodies are recognizing a common antigenic site.

10 The published amino acid analysis of the VP6 of bovine, simian and human rotaviruses were employed, along with proteolytic digests and chemical cleavage of the bovine rotavirus protein, in order to identify the antigenic site recognized by our monoclonal antibodies.
15 The intact VP6 is 397 amino acids in length (Figure 2). The smallest antibody-reactive fragment of VP6 had a molecular weight of approximately 6,300 (6.3K) and was comprised of 57 amino acids at positions 40 to 97. The results further indicated that within the 6.3K fragment,
20 the putative subgroup-specific site lies between amino acids at positions 40 to 60.

c) Virus Protein 3 (VP3)

 The outer shell polypeptide of rotavirus, VP3, is a protein comprised of 776 amino acids with an approximate
25 molecular weight of 82,000 (82K) in its unreduced form and 84,000 (84K) in its reduced form (Figure 3). It has also been shown to be a major antigen responsible for inducing neutralizing antibodies to the virus. This protein also has the ability to hemagglutinate red blood cells and is
30 responsible, in combination with VP7, in determining virus serotype.

 The initial steps in rotavirus replication involve two major events; attachment of the virus particle to target cells and the penetration/uncoating of the virus
35 particle within the cell. The enzyme trypsin enhances

virus infectivity. However, this enhancement appears to act after adsorption, since trypsin does not affect the efficiency or rate of virus attachment to cells but does increase the levels of uncoated particles found in cells.

5 The molecular mechanism for trypsin enhanced infectivity occurs via the cleavage of the VP3 protein which has a molecular weight of approximately 84,000, into two fragments with approximate molecular weights of 28,000 and 60,000. Therefore, the trypsin cleavage site of VP3 is

10 important in rotavirus replication.

The 776 amino acids making up VP3 of bovine rotavirus have been sequenced. A partial amino acid sequence of simian VP3 has also been determined (Figure 3). Comparison of the amino acid sequences of these VP3

15 proteins illustrates that the peptide fragment mimicking the trypsin-cleavage site (box in Figure 3) is conserved between these two isolates of the virus. It is likely that this region is conserved among most of the rotaviruses since most rotaviruses require trypsin to

20 enhance their infectivity.

We have synthesized the peptide corresponding to the trypsin cleavage site shown in Figure 3. The synthetic peptide exhibits three significant characteristics.

25 First, it stimulates the formation in animals of antibodies which neutralize the virus.

Second, it is able to bind to bovine rotavirus via binding to VP6 monomeric and oligomeric protein units. A practical application of this type of binding

30 can be foreseen with respect to using the VP6 protein as a carrier in order to enhance immune responses to this synthetic peptide and perhaps to other synthetic peptides which contain the consensus binding structure. Since the interaction between the VP6 protein and the peptide is

35 able to withstand harsh treatments, such as boiling in

sodium dodecyl sulfate, it may be suitably stable in vaccine preparations. Also, using a carrier which has been shown to be an antigenic and highly immunogenic rotavirus protein increases the chances that better
5 protection against disease can be achieved by using less of the immunogen in a vaccine preparation.

The third important feature of the synthetic peptide is that it can be cleaved by trypsin thereby demonstrating that it behaves like the authentic VP3
10 protein in this respect. Since the synthetic peptide can effectively compete with the natural 84,000 protein for trypsin, the rate of infection can be inhibited to some extent. Due to this fact, the synthetic peptide may also be valuable as a therapeutic agent against neonatal
15 gastroenteritis.

In this specification, as is conventional in the art, an amino acid position in a given sequence is assigned a number equating to the number of amino acid residues in the sequence, counting from the N terminal to
20 that position. In considering synthetic sequences, analogous to a natural polypeptide or to part of a natural polypeptide, it is generally convenient to base the numbering system on the natural polypeptide and to use that system for numbering the synthetic sequence, even
25 though the synthetic sequence may be different from the natural sequence.

In other systems, short synthetic peptides corresponding to fragments of particular virus proteins have been shown to carry antigenic properties of the
30 intact protein. Thus, synthetic antigens have been used for provoking antibodies against a variety of viruses including influenza, polio encephalomyelitis, foot and mouth disease and hepatitis B. We have now demonstrated similar properties for synthetic peptides of rotaviruses.
35

SUMMARY OF THE INVENTION

The present invention is based on our discovery that peptides of rotavirus VP7, VP6 and VP3 possess important biological activities. The 14 K peptide of VP7, and shorter peptides within it comprising neutralizing amino acid sequences (epitopes), demonstrate at least a substantial portion of the immunogenic activity of the entire rotavirus glycoprotein or the rotavirus itself. Similarly, a 6.3K fragment of VP6 and shorter peptides within it also have neutralizing properties. The invention further relates to a synthetic peptide of VP3 which corresponds to the trypsin cleavage site of that protein and which has three important characteristics: 1) it stimulates the formation of neutralizing antibodies to the virus; 2) it binds to the virus particle via the VP6 protein; and 3) it mimics the trypsin-cleavage site in that it can be cleaved by trypsin and therefore can compete with virus for trypsin, thereby interfering with infectivity.

The short peptides (epitopes) can be synthesized directly or produced as fusion proteins, whereas the 14 K and 6.3 K peptides can be produced only as fusion proteins. Being produced as heterologous proteins, the peptides are essentially free of other proteins of viral origin and of the infectious agents itself and, therefore, can be used as vaccines without fear that the disease may be transmitted. Furthermore, these peptides are free of other protein material of mammalian origin.

The present invention, therefore, comprises immunogenic fusion polypeptides and synthetic peptides having amino acid sequences which correspond to at least a portion of the sequence of rotavirus VP7, VP6, and VP3. All of these peptides display a degree of immunogenicity sufficient to stimulate production of neutralizing rotavirus antibodies. Therefore, the invention is

directed to compositions comprising the peptides and particularly compositions useful as vaccines and to the use of such vaccines to immunize avian and mammalian species against rotavirus infection. Furthermore, the peptide of VP3 can compete with the virus for trypsin and in this way interfere with virus infectivity thus acting as a therapeutic agent.

The first step is the production, by synthetic or recombinant DNA methods, of peptides corresponding to antigenic fragments of rotavirus VP7, VP6, or VP3. The peptides are then attached to suitable carriers and the resulting products are used as vaccines. Vaccination leads to the production of neutralizing rotavirus antibodies and the protection against infection.

Peptides having the following amino acid sequences stimulate the formation of rotavirus neutralizing antibody.

a) Virus Protein 7 (VP7)

Peptide 165-295 The amino acid sequence of the 14 K fragment of bovine rotavirus is shown in the box in Figure 1. This 14,000 molecular weight (14 K) fragment, spanning amino acids 165 to 295, was found to be responsible for much of the immunological activity of intact VP7 and was shown to be capable of eliciting antibodies which neutralized the virus and blocked viral attachment to host cells. Due to its large size (130 amino acid residues), the 14 K fragment comprises a plurality of antigenic determinants, several of which are in regions that undergo sequence changes or variability in different strains of rotavirus (variable strain-specific regions) and others in regions which are common or highly conserved among the various strains (conserved regions). Peptides corresponding to both types of regions can be used for the preparation of vaccines and include the following.

Peptide 174-183 Try-Gln-Gln-Thr-Asp-Glu-Ala-Asn-Lys

Peptide (178-181) - (251-259) Asp-Glu-Ala-Asn-Lys-Lys-Leu-Gly-Pro-Arg-Glu-Asn-Val-Ala

Peptide 247-259 Arg-Asn-Cys-Lys-Lys-Leu-Gly-Pro-Arg-Glu-Asn-Val-Ala

5 Peptide 275-295 Pro-Thr-Thr-Ala-Pro-Gln-Thr-Glu-Arg-Met-Met-Arg-Ile-Asn-Trp-Lys-Lys-Trp-Trp-Gln-Val

b) Virus Protein 6 (VP6)

Peptide 40-97 Thr-Met-Asn-Gly-Asn-Glu-Phe-Gln-Thr-Gly-Gly-Ile-Gly-Asn-Leu-Pro-Ile-Arg-Asn-Trp-Asn-Phe-Asn-Phe-Gly-Leu-Gly-Thr-Thr-Leu-Leu-Asn-Leu-Asp-Ala-Asn-Tyr-Val-Glu-Thr-Ala-Arg-Asn-Thr-Ile-Asp-Tyr-Phe-Val-Asp-Phe-Val-Asp-Asn-Val-Cys-Met.

15 This 6,300 molecular weight (6.3K) fragment spanning amino acids 40 to 97 (shown in the box in Figure 2) reacted with monoclonal antibodies which immunoprecipitated and neutralized the intact virus. Within this span of 57 amino acids, a shorter sequence also stimulates the formation of neutralizing antibodies and hence is useful as a vaccine. The amino acid sequence of this shorter peptide is as follows:

Peptide 40-60 Thr-Met-Asn-Gly-Asn-Glu-Phe-Gln-Thr-Gly-Gly-Ile-Gly-Asn-Leu-Pro-Ile-Arg-Asn-Trp-Asn.

c) Virus Protein 3 (VP3)

25 Peptide 232-256 Asn-Ile-Ala-Pro-Ala-Ser-Ile-Val-Ser-Arg-Asn-Ile-Val-Tyr-Thr-Arg-Ala-Gln-Pro-Asn-Gln-Asp-Ile-Ala

This peptide spans the trypsin cleavage site of VP3. Its location within the amino acid sequence of VP3 is shown in the box in Figure 3.

30 The 14K and 6.3K polypeptides and their component peptides of VP7 and VP6 respectively, and the peptide corresponding to the trypsin cleavage site of VP3, of this invention may be used for prevention and control, or diagnosis of rotavirus infections of birds and animals including man. These peptides, and combinations and

modifications of them, attached to carriers known in the art, are effective as vaccines for either passive or active immunization. Further, the peptide corresponding to the trypsin cleavage site of VP3 may serve as a
5 therapeutic agent to block or interfere with virus infection.

In accordance with the invention, it was discovered that neutralizing antibodies in laboratory animals are induced by these peptides covalently linked to
10 keyhole limpet hemocyanin. Other carriers known in the art can also be used to make the conjugate with the peptide sequences of the invention including any natural or synthetic carrier. The term "carrier" is a recognized term in the art and literature and sometimes is referred
15 to as "coupler", "protein carrier" or "hapten carrier". Natural carriers used in accordance with the invention are known and are typically keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), ovalbumin (OVA), Beta-galactosidase or penicillinase. Synthetic carriers
20 are typically multi-poly-DL-alanyl-poly-L-lysine and poly-L-lysine. In addition, it was discovered that the synthetic peptide corresponding to the trypsin cleavage site of VP3 could itself stick to VP6 in a variety of forms. Therefore, VP6 could also serve as a carrier for
25 the synthetic peptide corresponding to the trypsin cleavage site of VP3. This leads to the additional use of incorporating the amino acid sequence of the trypsin cleavage site of VP3 into a variety of macromolecules and binding them via this site to VP6 thereby increasing the
30 immunogenicity of the macromolecules.

Generally, the peptide sequences of the invention are covalently attached to the molecule of the larger compound or, especially where it and the larger compound are constructed together by recombinant DNA technology,
35 the sequences of the invention may be linked in some other

manner to the larger compound. For example, where the larger compound is a polypeptide, such as Beta-galactosidase or penicillinase, it may be interposed in the amino acid chain of that compound. Further, the peptides may be synthesized in multiple repeats by recombinant DNA technology to produce a polypeptide of repeating epitopes containing the immunogenic region.

The invention also encompasses biologically active compositions comprising the peptides (antigens) and an immunostimulant or adjuvant and wherein the peptides are administered with the immunostimulant. Complete Freund's adjuvant, aluminum hydroxide and liposomes are examples of such immunostimulants. Other natural and synthetic immunostimulants are well known in the art. The administration of antigen and adjuvant need not be concurrent; one may proceed the other, in part or all of it.

The peptides of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the polypeptides hereof are combined in admixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation are described in Remington's Pharmaceutical Science by E.W. Martin, which is hereby incorporated by reference. Such compositions will contain an effective amount of the polypeptide product hereof, together with a suitable amount of carrier vehicle in order to prepare pharmaceutically acceptable compositions suitable for effective administration to the host. One preferred mode of administration is parenteral. Suitable animal health formulations are prepared, as in the case of pharmaceutical compositions, mutatis mutandis.

The vaccines of the present invention, incorporating a suitable polypeptide or peptide produced

as herein described, can be prepared according to known methods, wherein said polypeptide or peptide is combined in admixture with a suitable vehicle. Suitable vehicles include, for example, saline solutions, various known
5 adjuvants or other additives recognized in the art for use in compositions applied to prevent viral infections. Such vaccines will contain an effective amount of the polypeptides or peptides hereof and a suitable amount of vehicle in order to prepare a vaccine useful for effective
10 administration to the host. Attention is also directed to New Trends and Developments in Vaccines, editors: A. Voller and H. Friedman, University Park Press, Baltimore, 1978, which is hereby incorporated by reference for further background details on the preparation of vaccines.

15 The peptides can be prepared according to any conventional method of synthesizing peptides. Either solid phase processes or liquid phase processes may be employed. Such processes for synthesizing peptides are described in, for example, "The Peptides", volume 2
20 (1984), E. Gross and J. Meinhafer Editors, Academic Press, New York, U.S.A.

Also, it is possible to clone a DNA sequence corresponding to these amino acid sequences into vectors known in the art, for example pBR 322, since this plasmid
25 readily accepts short lengths of DNA, and growth in, for example E coli HB101 or other hosts known in the art, would allow expression of the poly-peptide, which could be further enhanced by inclusion of promoters in the DNA sequence and other published procedures. Therefore, the
30 invention is further directed to replicable DNA expression vectors which contain gene sequences which encode for the polypeptides in expressible form. The invention is also directed to recombinant host cells such as microorganism, strains or cell lines transformed with expression vectors
35 known in the art and to the cultures thereof.

It is clear that all of the sequences which are given herein are by way of example only and that other composition related to relevant antigenic determinants or sequences in which limited conservative amino acid changes are introduced, can also be used.

DETAILED DESCRIPTION OF THE INVENTION

a) The 14 K Polypeptide of VP7 To prepare the various antigens to be used for immunization, Bovine Rotavirus (isolate C486, subclone 13), was propagated in MA-104 cells and purified by centrifugation. One milligram of purified double-shelled virus was then fractionated on a 10% preparative polyacrylamide gel. The 38.2 K glycoprotein was localized in the gel by staining side strips of the gel with Coomassie blue. To extract the glycoprotein, gel strips were subjected to electroelution.

The 14 K polypeptide fragment was prepared by placing a gel strip containing the 38.2 K glycoprotein (VP7) into the well of a 5% stacking-20% resolving polyacrylamide gel. A 13 cm X 1 cm gel strip was routinely treated with papain (Calbiochem-Behring, San Diego, Ca.) at a concentration of 130 ug/cm of gel; since this gave the best preparation of 14 K polypeptide as illustrated in Figure 4 (lane B). Electrophoresis and in situ enzyme digestion of the 38.2 K glycoprotein was performed according to the specifications of Cleveland et al (Cleveland, D.W., S.G. Fisher, Kirschner and U.K. Laemmli. 1977. Peptide mapping by limited proteolysis in SDS and analysis of gel electrophoresis. J. Biol. Chem. 252: 1102-1106) and prestained molecular weight markers were used in order to visualize the time of maximum resolution between the 14.3 K and 18.4 K markers. Further localization of the 14 K polypeptide was achieved using the molecular weight markers. The peptide fragment was then electroeluted from the gel slices, and a protein

determination performed. Confirmation of the authenticity and purity of the peptide was by examination of its profile on polyacrylamide gels (Figure 4, lane C) and by its reaction with monoclonal antibody from hybridoma 11D12-6 (Figure 4, lane D).

The electroeluted 14 K polypeptide was then lyophilized and a portion of the preparation was conjugated to bovine serum albumin (BSA) as follows. One milligram of peptide was first dissolved in 125 ul of 0.1 M PBS pH 7.4. The BSA solution was prepared by dissolving 1.25 mg BSA in 600 ul 0.1 M PBS pH 7.4 and to this were added dropwise 250 ul of a 2.5 M gluteraldehyde solution and the peptide solution consecutively over 15 minutes. The reaction mixture was gently agitated for 24 hours at room temperature and then dialyzed extensively against sterile distilled water. Lyophilization of the conjugated peptide yielded a pinkish powder which was stored in dessicant at -20°C.

Once all the antigens were prepared, groups of ten mice (Charles River, Wilmington, MA) were immunized with either the BSA-unconjugated polypeptide, BSA-conjugated polypeptide, infectious double-shelled virus or purified VP7 according to the protocol outlined in Table 2. The quantities of antigen to be administered were determined on an equimolar basis. Antibody responses to the different antigens were characterized by ELISA and immuno-blot ELISA using bovine rotavirus (isolate C486 subclones 12 and 13) as the antigen and by serum neutralization assays.

As illustrated in Figure 5 (upper panel) there was a significant antibody response to all the antigens used. Noteworthy is the similarity in response between VP7 (38.2 K glycoprotein) and the 14 K polypeptide fragment. It also appears that conjugation of the 14 K polypeptide to a carrier was not necessary to induce a

good antibody response. This may be due to the large size of the polypeptide fragment thereby increasing the probability of it containing both B-cell and T-cell determinants.

5 The animals immunized with the 14 K fragments were boosted at 61 days with infectious, double-shelled virus. This was done in order to investigate the possibility of the fragments priming an immune response. Even though, after analysis of all the sera, the animals
10 did show a good antibody response to the peptides, they also demonstrated an additional, albeit minor, response after the infectious virus was administered (68 days). These results were encouraging since they illustrated that the 14 K polypeptide alone was capable of inducing a
15 respectable antibody titer. More importantly, these antibodies had neutralizing ability. Sera from groups A-D possessed neutralizing antibodies (Figure 5, lower panel), with the best response produced by animals immunized with infectious virus (Group D). Total antibody titers as
20 measured by ELISA and neutralizing antibody titers were similar for both the conjugated and unconjugated form of the polypeptide. At 68 days, after all the groups had been exposed to infectious virus, the neutralizing antibody titer increased slightly over that seen at 51
25 days suggesting that each subsequent exposure further stimulates the immune response, or alternatively, there may be other antigens on the infectious virus that are capable of inducing a neutralizing response. The most likely candidates for such antigens are the minor outer
30 shell protein (VP3, 84 K mol. wt.) and the major inner shell protein VP6 (45 K mol. wt.). Several reports have indicated that both of these proteins are capable of inducing neutralizing antibodies, although to a much lesser extent than the major glycoprotein. This, in fact,
35 is supported by the presence of antibodies to the 45 K

protein as illustrated by the immuno-blot ELISA reactions at 68 days even though antibodies to the 84 K protein could not be detected (Figure 6). As will be discussed further below monospecific and monoclonal antibodies to the 45 K antigen have demonstrated neutralizing ability.

Immuno-blot ELISA reactions of sera from selected animals in each group at 37, 51 and 68 days are shown in Figure 6. All the sera, except those obtained prior to immunization and the negative control group (E), possessed antibodies to VP7. Anti-peptide antibodies, although produced to the 14 K polypeptide prepared from only one of the glycoprotein species present in bovine rotavirus isolate C486, reacted with both glycoprotein species present in the protein profile of the parent isolate C486. This was significant since electrophoretic analysis of the genomic RNA from the two subclones of isolate C486 demonstrated a difference in mobility of the corresponding genes coding for this glycoprotein. However, it appears that despite this genetic heterogeneity, the 14 K polypeptide is to a large extent, conserved between the two glycoprotein species of BRV isolate C486 subclones 12 and 13. Another interesting observation was the similar intensity displayed by the reaction of the glycoprotein species with anti-peptide antibodies suggesting that the 14 K polypeptide may represent an immunodominant region of the glycoprotein.

The Component Peptides Within The 14 K Polypeptide

In order to be able to synthesize appropriate peptides it was necessary to localize the 14 K molecular weight polypeptide fragment within VP7. The following characteristics of the 14 K polypeptide were taken into consideration to accomplish this localization. The 14 K peptide has i) a carbohydrate moiety, ii) extensive disulfide bridging, iii) relatively conserved region(s) among different rotavirus serotypes, iv) hydrophilic

areas; and v) potential immunogenic regions. In addition, the cleavage patterns obtained by partial proteolysis of the glycoprotein using chymotrypsin, Staph. aureus V8 protease, papain and cyanogen bromide aided in locating
 5 the 14 K polypeptide fragment (Figure 7).

The amino acid sequence of Nebraska calf diarrhea virus (NCDV bovine rotavirus), which exhibits high nucleic acid homology with the C486 bovine rotavirus and is of the same serotype, was used to map the 14 K polypeptide
 10 fragment to the region spanning amino acids 165-295. A hydrophilicity plot of the corresponding NCDV glycoprotein identified several hydrophilic regions within this area. Based on this, four peptides, corresponding to amino acid residues 174-183, 178-181 and 251-259 spliced together,
 15 247-259, and 275-295 on VP7 of bovine rotavirus were synthesized by the solid phase peptide synthesis method of Merrifield.

The specific amino acid sequence of each peptide is as follows.

- 20 Peptide 175-183 Try-Gln-Gln-Thr-Asp-Glu-Ala-Asn-Lys
Peptide (179-183) - (251-259) Asp-Glu-Ala-Asn-Lys-Lys-Leu-Gly-Pro-Arg-Glu-Asn-Val-Ala
Peptide 247-259 Arg-Asn-Cys-Lys-Lys-Leu-Gly-Pro-Arg-Glu-Asn-Val-Ala
 25 Peptide 275-295 Pro-Thr-Thr-Ala-Pro-Gln-Thr-Glu-Arg-Met-Met-Arg-Ile-Asn-Trp-Lys-Lys-Trp-Trp-Gln-Val

The purity of each peptide was assessed using thin layer chromatography and reverse phase high performance liquid chromatography. Fast atom bombardment
 30 mass spectrometry was used to confirm molecular weights.

The reactivity and specificity of the synthetic peptides was determined by several methods.

- 1) ELISA with anti-VP7 monospecific serum, indicating specificity of the peptides for VP7 (Table 3).
- 35 2) ELISA with monoclonal antibodies specific for the neutralizing glycoprotein (VP7) and which had the ability

to block virus attachment, indicating specificity of the peptides for different regions or epitopes of VP7 (Table 3).

- 3) Adsorption blocking assay indicating that the two peptides, 247-259 and 275-295, blocked virus attachment to MA-104 cells, whereas the other two peptides did not (Figure 8).

The immunogenicity of the synthetic peptides was demonstrated in mice as follows.

- Peptide 174-183 was conjugated to keyhole limpet hemocyanin (KLH) via a bis-diazotised tolidine linkage that produces N-terminally bound peptides. The other three peptides were conjugated to KLH via N-maleimidobenzoyl-N'-hydroxysuccinimide ester producing N-terminally bound peptide conjugates. Each peptide was administered to groups of 10 CD-1 mice according to the schedule outlined in Table 4.

- Each mouse in groups 1 to 4 was given 100 ug of each KLH-conjugated peptide in Freund's Adjuvant. Group 5 was given 25 ug of each of the four KLH-conjugated peptides in Freund's Adjuvant. Group 6 received 1.6 ug of infectious double-shelled rotavirus and Group 7 represented the negative control group which received saline plus Freund's Adjuvant. The antigen preparations were administered three times over a six-week period and mice were bled prior to each immunization.

- The reactivity and specificity of the antibodies elicited in mice by the polypeptides were reactive with complete rotavirus particles and individual corresponding peptides. Antibody titers to double-shelled rotavirus (upper panel - Figure 9) and to individual, corresponding peptides (lower panel - Figure 9) were determined by an enzyme-linked immunosorbent assay. All four synthetic peptides induced antibodies which reacted with rotavirus after the first immunization. Subsequent immunizations

resulted in an increased response in all groups. Anti-peptide antibodies to the individual corresponding peptides were also induced after the first immunization indicating that all KLH-peptides were immunogenic (lower panel).

The mouse antibodies against two of the peptides were also capable of inhibiting the infectivity of the virus, as determined in vitro in plaque reduction assays performed as follows.

Neutralization of bovine rotavirus isolate C486 by mouse antisera was determined by a standard 50% plaque reduction assay. Virus dilutions representing 30-50 PFU were mixed 1:1 with various dilutions of antibody and incubated for 1 h at 37°C. Virus adsorption to MA-104 monolayers was allowed to proceed at 37°C for 2 h before the virus inoculum was removed; the cells were washed with MEM and then overlaid with 1.6% Bacto-agar (Difco) diluted in MEM and supplemented with 5 ug of pancreatin per ml, 0.7% of a 1:1,000 neutral red stock solution, and 0.1% DEAE-dextran. Plaques appeared after 5 to 6 days of incubation at 37°C.

As is shown in Figure 10, peptide 247-259, peptide 275-295 and the mixture of the four peptides induced virus neutralizing antibodies which increased after each immunization. This indicates that antibodies produced by these two polypeptides react with viral epitopes involved in virus attachment.

To demonstrate protection, a passive antibody transfer experiment was carried out using monoclonal antibody 10D2-7 which specifically recognizes synthetic peptide 275-295 (Table 5). Four groups each consisting of 10, 7 day old mice were first separated from their mothers for 2 hours and then given the appropriate Time 0 preparations by tubing to the stomach. Approximately 1 hour later they were given the Time 1 preparation. The

mice were kept at 33°C for 8 hours with constant monitoring of fecal consistency. After 8 hours, the mice were sacrificed and their intestines removed and pulverized. The amount of infectious virus in the intestine was determined by 50% plaque-reduction assay.

As shown in Table 5, mice in Group III which did not receive monoclonal antibody were not protected. They became diarrheic and had 5 logs of rotavirus in intestinal homogenates prepared 8 hours after challenge. There was a significant reduction in the amount of diarrhea and in the level of virus in intestinal homogenates of mice in Group I (given monoclonal antibody orally before challenge with virus) and Group II (challenged with a mixture of monoclonal antibody and virus).

Further evidence of the immunogenicity and protective capacity of these VP7 peptides is presented below after the section b) on VP6.

b) The 6.3K. Polypeptide of VP6

Four monoclonal antibodies to the bovine rotavirus (isolate C486) VP6 (45K nucleocapsid protein) were identified via immunoprecipitation of infected cell lysates (Figure 11) and by an immuno-blot ELISA (Figure 12). The four monoclonal antibodies (1D7, 1B4, 1B9, 1D10) demonstrated neutralizing ability similar to that observed for monospecific antiserum VP6, although lower than that exhibited by antisera to the two outer capsid proteins VP7 and VP3 (Table 6). In addition, a mixture of these four monoclonal antibodies recognized the nucleocapsid protein of a porcine (OSU), bovine (NCDV, UK) and monkey (SA11, RRV) rotaviruses belonging to sub-group 1, and human (WA, ST4) rotaviruses belonging to sub-group 2 in an immunoblot-ELISA (Figure 13).

In order to localize the antigenic determinants recognized by these monoclonal antibodies, immuno-blot ELISA reactions were carried out on nitrocellulose-blotted

partial protein digests of VP6 using monoclonal antibodies and monospecific antiserum (Figure 14). Examination of the digests of each specific enzyme revealed that all four monoclonal antibodies recognized essentially the same peptides of VP6 in that digest. In the case of chymotrypsin and *S. aureus* V8 protease digests, the reactivity of monospecific serum was identical with that demonstrated by the monoclonal antibodies. Figure 15 illustrates the digestion pattern and antibody reactivity pattern of VP6 generated by chemical cleavage with cyanogen bromide (CNBr) and carboxypeptidase. As indicated by the arrows, the smallest antibody-reactive CNBr generated fragment had a molecular weight of approximately 6,300. Computer analysis of gene 6 translated sequence (Figure 2; Taken from Estes, M.K., B.B. Mason, S. Crawford and J. Cohen, 1984. Cloning and nucleotide sequence of the simian rotavirus gene 6 that codes for the major inner capsid protein. *Nucleic Acids Research* 12:1875-1887) indicated that the only two possible cyanogen bromide fragments that could react with our monoclonal antibodies are: from amino acids 40-97, molecular weight 6,302.7; and from amino acids 300-365, molecular weight 6,830 (Table 7). Antibody reactivity with the carboxypeptidase digest of VP6 indicated that the antigenic site is located at the amino terminal end of the molecule within the first 79 amino acids since a 9,500 molecular weight fragment was immune reactive, whereas a 6,000 molecular weight fragment (50 amino acids) was not (Figure 15, lane 1). Hence, the 6.3K polypeptide fragment responsible for the neutralizing activity is comprised of the 57 amino acids in position 40 to 97. These are enclosed in the box in Figure 2.

The Component Peptides of 6.3K of VP6

Using similar methods to those described above for the peptide fragments of the 14K polypeptide of VP7,

the amino acid residue 40 to 60 of the 6.3K fragment of VP6 was synthesized.

The specific amino acid sequence of the peptide is as follows:

5 Peptide 40-60 Thr-Met-Asn-Gly-Asn-Glu-Phe-Gln-Thr-Gly-
Gly-Ile-Gly-Asn-Leu-Pro-Ile-Arg-Asn-Trp-Asn-Gly-Cys-OH

The terminal Gly-Cys-OH structure is not part of the antigenic fragment but was added to our sequence to provide a linkage site.

10 The reactivity and specificity of the synthetic polypeptide was determined by several methods.

1) ELISA with anti-VP6 monospecific serum, indicating specificity of the peptide for VP6 (Table 8).

15 2) ELISA with monoclonal antibodies specific for the neutralizing protein (VP6), indicating specificity of the peptide for the epitope of VP6 (Table 8).

3) Immunoblot ELISA with monoclonal antibodies, indicating specificity of the peptide for the epitope of VP6.

20 The immunogenicity and protective capacity of the peptides of VP6 and VP7 are further illustrated by the following examples.

25 The peptides corresponding to 275-295 of VP7 and 40-60 of VP6 were synthesized using Fmoc protected amino acids and solid phase peptide synthesis (Cambridge Research Biologics, Cambridge, England; IAF Biochemical, Laval, Quebec, Canada).

30 The peptide-Keyhole Limpet Hemacyanin (KLH) conjugates were produced using N-maleimido-benzoyl-N'-hydroxysuccinimide (MBS) ester derivitized KLH and a cysteine added to the N terminal of the peptide (Cambridge Research Biologics, Cambridge, England) or water soluble carbodiimide. The peptides were also conjugated to the E coli pilin protein K99. Urea extracted and ammonium
35 sulfate precipitated K99 was purified and the peptides

31.

conjugated to it using the carbodiimide method. This protocol yielded a conjugate with a peptide to K99 ratio of 3.5:1 as determined by ultraviolet spectroscopy and amino acid analysis and confirmed by gel electrophoresis.

5 Following synthesis of peptides and conjugates the products were assessed for immunoreactivity using an immunoblot ELISA assay developed with polyclonal anti-rotavirus serum.

10 The ability of the synthetic peptide 275-295 of VP7 coupled to a K99 carrier to boost antibody levels in cows prior to parturition was tested. As illustrated below both cows had antibodies to rotavirus prior to immunization, and these titers did not increase after vaccination with the K99 carrier protein alone. After one
15 immunization with K99 coupled to the synthetic peptide administered in DDA - aluminum hydroxide gel (100 ug of conjugate) the antibody response was increased by 1.0 - 1.5 logs.

Antibody Titers to Rotavirus as Determined by ELISA

20

	Prebleed	After K99 Alone	After K99 Plus Synthetic Peptide 275-295 of VP7
25 Cow 1	8,000	8,000	100,000 ^a
Cow 2	10,000	10,000	100,000

30

35

In a second experiment mice were immunized with 100 ug of either the KLH-peptide or the K99-peptide conjugates in Freund's Complete Adjuvant and the anti-rotavirus immune responses were monitored. The amount of the conjugates used in the immunizations contained approximately equal quantities of each of the rotavirus peptides. No difference in the response of mice to the conjugates was seen over 3 immunizations (Table 9). Although the two carriers differ greatly in molecular weight, KLH being approximately 3.5×10^6 and K99 approximately 19,000 daltons, they appear to be equally effective in generating antibodies to these peptide haptens.

In addition to the use of carriers in producing immune responses to peptides, many adjuvants have been investigated to further increase these responses. Unfortunately, most adjuvants that are powerful enough to increase the immune responses are not acceptable for use in humans or animals. The adjuvant chosen for use in this study was the quaternary amino surfactant dimethyl dioctadecylammonium bromide (DDA). This adjuvant was selected because of its reported abilities as a powerful adjuvant toward haptens and the fact that it is approved for use in food animals. We used the K99-VP7 275-295 conjugate to compare effects of DDA and Freund's Complete Adjuvant.

Following immunization, both the anti-rotavirus titer and the anti-K99 activity found in the immunized mouse serum was measured. Each mouse received 100 ug of conjugate with either 0.1 ml FCA or 100 ug of DDA. Following two immunizations there was a significant increase in the rotavirus antibody titers in both groups (Table 10). The levels of rotavirus antibodies seen in this experiment approximate those seen following a protective immunization schedule using live rotavirus (Table 11).

We then examined the dose of antigen needed to produce this protective response. We used three doses of K99-VP7 peptide conjugate (1, 10 and 100 ug/mouse) combined with the adjuvant DDA. As shown in Table 11 the conjugate produced dose related responses to the carrier protein K99, but only the 100 ug dose approached the anti-rotavirus antibody level produced by virus-immunized controls.

In general "free" synthetic peptides generate only low titered, short duration responses. These responses have involved the use of strong adjuvants of limited practical usefulness. However, peptides which form aggregates, either spontaneously or through the use of lipid side chains, have produced functional titers. Alternatively, peptides have been used to prime the immune system and produce a secondary type of response when immunized with a suboptimal dose of parent protein. We have investigated several methods used to produce responses to other peptides. These methods include: 1) associating the peptides with liposomes containing an adjuvant, 2) attempting to aggregate the peptides with the adjuvant DDA, and 3) using peptides in combination with FCA to prime the immune response.

The data gathered from the previous studies have shown that synthetic peptides conjugated to carriers could produce levels of anti-rotavirus antibodies in the serum of mice that is similar to the titers seen following live virus inoculation. This encouraged us to use a rotavirus mousa model system for rotavirus diarrhea to demonstrate protection of neonates from disease challenge. Female mice were immunized three times during a schedule of breeding and pregnancy. The last immunization was given 2 weeks prior to whelping. Each of the carriers and synthetic peptides was used in combination with FCA. The K99-conjugates were also used with DDA (Table 12). The

mouse pups were allowed to suckle and were challenged at 7 days of age with a bovine strain of rotavirus. The effectiveness of the immunizations was determined following challenge of the neonates in a single blind experiment. Morbidity, mortality and severity of diarrhea were scored over a 48 hr period following the challenge. Most diarrhea and morbidity was apparent within 3-5 hrs following challenge.

All synthetic peptide preparations provided a substantial reduction in morbidity, and two preparations provided protection equal to the whole virus. In this experiment K99 did not work as well as KLH as a carrier but peptide-K99 conjugates still provided protection to the challenge.

c) The Trypsin Cleavage Site of VP3

The amino acid sequence of the potential trypsin-cleavage site of VP3 was chosen based on the following facts: 1) the size of the fragments resulting from trypsin cleavage of the authentic VP3 protein are known, 2) the amino acid sequence of the authentic VP3 is known (Figure 3) and, 3) the cleavage specificity for the enzyme trypsin is known. The specific amino acid sequence of the cleavage site is as follows: Asn-Ile-Ala-Pro-Ala-Ser-Ile-Val-Ser-Arg-Asn-Ile-Val-Tyr-Thr-Arg-Ala-Gln-Pro-Asn-Gln-Asp-Ile-Ala. This peptide has a molecular weight of 2,753 and represents amino acids 232 to 256 of VP3.

Figure 16 illustrates that antisera to the synthetic peptide reacted specifically with both the reduced (denoted 1 and 1') and unreduced (denoted 4 and 4') VP3. Since it specifically reacted with the lower band of the closely migrating 84,000 doublet (denoted as 1) this unequivocally identifies it as the product of gene 4 and ensures that the synthetic peptide corresponded to this site on authentic VP3.

Since the trypsin cleavage site is a biologically important region it was important to determine whether

antiserum to this peptide could neutralize virus infectivity. As illustrated in Table 13 below, antiserum to the synthetic peptide as well as monoclonal antibodies specifically reacting with the peptide were able to effectively neutralize virus infectivity.

Table 13 Neutralizing Ability of Antisera to the 232-256 Synthetic Peptide of VP3

10	Antibody	Virus Neutralizing Titre Determined By 50% Plaque Reduction Assay
	Antiserum to Synthetic Peptide	5,000
15	Monoclonal Antibody to Synthetic Peptide	10,000

When 100 ug of the synthetic peptide was reacted with 2.0 ug of purified virus for 30 min at 37°C a laddering of VP6 (the 45K nucleocapsid protein) was observed (Figure 17, lane C). At lower concentrations of the peptide, i.e. 25 ug, the laddering was not obvious (lane A). A corresponding ladder to that at the 45K location was also observed at the 90K and 135K regions (lane C). Support for the binding of the peptide to VP6 was provided by the fact that the molecular weight increments in the ladder correspond to the molecular weight of the synthetic peptide monomer. In addition, trypsin-treatment of the virus-peptide complex (lanes B and D) reduced the ladder in both the nucleocapsid monomer (45K), dimer (90K) and trimer (135K) to the extent that the virus profile was almost identical to the uncomplexed, trypsin-treated virus profile (lane E).

Definitive proof that the peptide bound to VP6 was demonstrated by the fact that a ladder was detected in

both the 45K and 90K region with antisera produced against the synthetic peptide (Figure 18, lane C). Reactivity was not observed however, when the virus was reacted with the synthetic peptide and then treated with 0.96 ug of trypsin (Figure 18, lane B) indicating that even when it is bound to the VP6 protein it maintains the trypsin-cleavage site.

Reactivity of the 232-256 VP3 synthetic peptide with VP6 was maintained under conditions where samples were treated with urea sample buffer for 30 min at 37°C (Figure 19, lane A) and when samples were treated with Laemmli buffer without B-mercaptoethanol (BME) but with boiling (lane B). However, when BME was included in the sample buffer and the sample was boiled, prior to electrophoresis, the ladders in both the 45K and 90K regions (indicated by arrowhead) disappeared (lane C), suggesting that secondary structure specified by disulphide bridging was necessary to maintain the VP6-synthetic peptide complex.

The synthetic peptide corresponding to the trypsin cleavage site of VP3 was also tested for its authenticity by reacting it with trypsin. Upon electrophoresis of the peptide, both monomeric and dimeric forms were observed after staining with Coomassie blue (Figure 20, lane G). The monomer, however, is not clearly visible since it quickly diffused out of the 17.5% resolving gel upon destaining. Treatment of the synthetic peptide with 9.6 ug (lane C) and 19.2 ug (lane A) of trypsin/100 ug of synthetic peptide for 30 min at 37°C reduced the amount of visible peptide by 90-100%. At a concentration of 0.96 ug of trypsin/100 ug of peptide, approximately 30% of the peptide remained uncleaved (lane E). The progressive cleavage of the peptide by increasing concentrations of trypsin indicates that the peptide is being specifically recognized by the enzyme at one of two potential sites.

Since we had established that the synthetic peptide could be recognized and cleaved by trypsin we investigated whether it could compete with the intact 84K VP3 for this enzyme. The extent of competition was measured by the ability of increasing concentrations of the synthetic peptide to reduce the amount of the authentic VP3 to be cleaved into its two products (molecular weights of approximately 60,000 indicated by lower arrowheads in lane B, and 28,000 indicated by upper arrowhead in lane F of Figure 21). The amount of trypsin used in this experiment was established such that the lowest quantity of synthetic peptide used, i.e. 25 ug of peptide and 25 ug of VP3 were completely cleaved (Figure 21, lane B). As the amount of synthetic peptide increased to 200 ug VP3 becomes evident (indicated by arrowhead in lane F), thereby attesting to the authenticity of the synthetic peptide.

Since the synthetic peptide could bind to the nucleocapsid protein and still be cleaved by trypsin we wanted to investigate whether it had any effect on virus infectivity. When increasing amounts of the synthetic peptide were mixed with infectious virus the number of visible plaques were reduced. At high concentrations (Figure 22, lanes E-H) of the synthetic peptide the plaques were only visible 5-6 days after plaques in the control well (B) were seen. These plaques however, were very small and diffuse and were not readily seen in wells E-H. Based on this information, the synthetic peptide may have a therapeutic, in addition to a prophylactic, application.

The dosage range for vaccination against rotavirus related diseases is from about 1-100 ug of peptide per kg of body weight. It has been found that this range provides an effective immune response for any of the peptides disclosed herein.

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It is possible to replace one or more of the amino acid residues in the above sequences by other amino acid residues, which replacement can be expected to have no major effect on antigenicity or immunogenicity. It is also possible to add or delete amino acid residues within or flanking these sequences without dramatically altering antigenicity or immunogenicity. Thus, antigenic and immunogenic equivalents of these sequences also form part of the present invention.

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Table 2

Table 2 Schedule for Mice Immunized with 14k Peptide, VP7 and Infectious Virus

Groups/Immunogen	D A Y S						
	1	8	31	37	44	51	61 ² 68
A. Unconjugated 14K	prebleed	13.6ug; I.P.; F.C. ¹	13.6ug; I.P.; F.I.	Bleed	13.6ug; I.P.; F.I.	Bleed	0.675ug; I.P.; F.I. Bleed
B. Conjugated 14K	"	13.6ug; I.P.; F.C.	13.6ug; I.P.; F.I.	"	13.6ug; I.P.; F.I.	"	0.675ug; I.P.; F.I. "
C. Glycoprotein VP7	"	4.5ug; I.P.; F.C.	4.5ug; I.P.; F.I.	"	4.5ug; I.P.; F.I.	"	0.675ug; I.P.; F.I. "
D. Infectious virus	"	0.675ug; I.P.; F.C.	0.675ug; I.P.; F.I.	"	0.675ug; I.P.; F.I.	"	0.675ug; I.P.; F.I. "
E. Negative control	"	saline; I.P.; F.C.	saline; I.P.; F.I.	"	saline; I.P.; F.I.	"	E ₁ -0.675ug; I.P.; F.I. "
							E ₂ -saline; I.P.; F.I.

The quantities given are per mouse (10 mice per group); I.P.-intraperitoneal; F.C.-Freund's complete adjuvant; F.I.-Freund's incomplete adjuvant

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Table 3

Table 3
Reactivity^a of Monoclonal Antibodies and Monospecific Serum
With Synthetic Peptides of the 14K Fragment of VP7

SYNTHETIC PEPTIDES			
	p174-183	p178-181-251-259	p247-259 p275-295
Monospecific anti-glycoprotein (VP7) serum	2,500 ^a	5,000	1,000 1,250
Monoclonal antibodies			
4B5-5	50	50	5,000 50
11D10-4	10	250	4,000 100
11D12-6	10	50	100 8,500
10D2-7	10	50	20 10,000

^a antibody titers were determined by ELISA and are expressed as the reciprocal of the dilution giving a 50% end point.

Table 4

Table 4. Immunization Schedule for Mice Injected with Synthetic Peptides

Group Designation	Within 14K of VP7						
	0	1	2	3	4	5	6
1 p174-183	Pre- bleed	-100ug; FC ^a	--	--	-bleed -100ug; FI	--	-bleed -100ug; FI
2 p(178-181)-(251- 259)	Pre- bleed	-100ug; FC	--	--	-bleed -100ug; FI	--	-bleed -100ug; FI
3 p247-259	Pre- bleed	-100ug; FC	--	--	-bleed -100ug; FI	--	-bleed -100ug; FI
4 p275-295	Pre- bleed	-100ug; FC	--	--	-bleed -100ug; FI	--	-bleed -100ug; FI
5 p174-183 p178-(181-251)-259 p247-259 p275-295	Pre- bleed	-25ug;FC	--	--	-bleed -25ug;FC	--	-bleed -25ug;FC
		-25ug;FC					
		-25ug;FC					
		-25ug;FC					
6 Infectious Virus	Pre- bleed	-1.6ug;FC	--	--	-bleed -1.6ug;FC	--	-bleed -1.6ug;FC
7 Controls	Pre- bleed	-saline;FC	--	--	-bleed -saline;FC	--	-bleed -saline;FC

^a FC = Freund's complete adjuvant; FI = Freund's incomplete adjuvant.

Table 5

Table 5. Passive Antibody Transfer of Monoclonal Antibody 10D2-7

Group Designation	Time 0 ^a Preparations	Time 1 ^a Preparations	Titer ^b (PFU/ml)	Diarrhea ^c
I	1:50 dilution of MAB 10D2-7	5 x 10 ⁶ PFU/ml mouse rotavirus	3.5 x 10 ² PFU/ml	+
II	1:50 dilution of MAB 10D2-7 was mixed with 5 x 10 ⁶ PFU/ml of mouse rotavirus 1 hour prior to administration to neonates	MEM	5 x 10 ¹ PFU/ml	-
III	MEM	5 x 10 ⁶ PFU/ml mouse rotavirus	4 x 10 ⁵ PFU/ml	+
IV	MEM	MEM	0	-

^a Time 0 and Time 1 preparations were made as described above. 100 ul of the preparations were administered by tubing to the stomach of each neonate.

^b PFU/ml = plaque-forming units of rotavirus per ml of intestinal homogenate.

^c Diarrhea was assessed by the color and consistency of the fecal material compared to the control groups (III and IV).

Table 6

TABLE 6 Neutralizing Antibody Titers to Whole Rotaviruses of Polyclonal and

Monoclonal Antibodies

Antibody Type	Neutralizing Titer
Polyclonal	
anti-45K (VP6)	500 ^a
anti-38.2K/41.9K (VP7)	200,000
anti-82K/84K (VP3)	5,000
Monoclonal to VP6	
1D7	500
1B4	500
1B9	800
1D10	500

^a Neutralizing titre determined by the reciprocal of antibody dilution necessary to produce a 50% plaque reduction. The antibody concentration was standardized based on ELISA titers.

Table 7

TABLE 7 Cyanogen Bromide Cleavage Fragments of VP6 Based on Translated Sequence of Estes et al.

Amino Acid Position		Molecular Weight	No. of Residues
From	To		
180	295	12,989.170	115
100	177	9,085.530	77
300	365	6,830.448	65
41	97	6,302.700	56
394	444	4,944.510	50
1	37	4,133.350	36
365	394	3,414.630	29
295	300	613.710	5
37	41	476.610	4
97	100	393.400	3
177	180	307.360	3

Table 8

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Table 8. Reactivity of Monoclonal Antibodies and Monospecific Serum withSynthetic Peptide of VP6P-40-60

Monospecific anti-VP6 serum

10,000

Monoclonal Antibodies

1D7

5,000

1B4

5,000

1B9

8,000

1D10

6,500

Table 9

**TABLE 9. THE EFFECT OF CARRIER ON THE RESPONSE TO VP7 275-295 PEPTIDE AND
VP6 40-60 PEPTIDE**

Peptide	Carrier	Anti-Rotavirus ELISA Titers Following Immunization ¹		
		2 Weeks Post	2 Weeks Post	2 Weeks Post
		1st Immunization	2nd Immunization	3rd Immunization
VP6 - 40-60	KLR	1,333	13,335	42,170
	K99	13,335	31,623	74,990
VP7 - 275-295	KLR	4,870	42,170	56,234
	K99	7,500	23,713	56,234

¹ All mice were seronegative at the time of first immunization.

Table 10

TABLE 10. THE EFFECT OF ADJUVANT ON THE RESPONSE TO K99- VP7-275-295 CONJUGATE

Adjuvant	ELISA Titers Following Immunization ¹			
	Two Weeks Post		Two Weeks Post	
	First Immunization		Second Immunization	
Freund's Complete Adjuvant	Anti-rota	9		6,310
	Anti-K99	31,600		50,120
Dimethyl dioctadecyl Ammonium Bromide	Anti-rota	89		7,495
	Anti-K99	251,200		10,000,000

¹ All mice were seronegative at the first immunization.

Table 11

**Table 11. EFFECT OF CONJUGATE DOSE ON THE ANTIBODY RESPONSE TO VP7 275-295
PEPTIDE AND TO THE K99 CARRIER PROTEINS**

Dose of Conjugate	ELISA Titer Following Immunizations ¹		
	2 Weeks Post		2 Weeks Post
	First Immunization	Second Immunization	
1 ug	Anti-rotavirus	1.5	2.5
	Anti-K99	63	4,500
10 ug	Anti-rotavirus	1.5	2.5
	Anti-K99	710	79,500
100 ug	Anti-rotavirus	89	7,495
	Anti-K99	251,200	10,000,000
(Virus Control)	Anti-Rotavirus		15,850
	Anti-K99		0

¹ All mice were seronegative at the first immunization.

Table 12

**Table 12. PROTECTION OF NEONATAL MICE FROM ROTAVIRUS CHALLENGE FOLLOWING 3
IMMUNIZATIONS OF DAMS WITH 100 UG OF IMMUNOGEN**

Immunogen	Adjuvant	Diarrhea Score
KLH	FCA ^a	4+
KLH (40-60) of VP6	FCA	1+
KLH (275-295) of VP7	FCA	-
K99 (40-60) of VP6	FCA	1+
K99 (275-295) of VP7	FCA	2+
K99 (40-60) of VP6	DDA ^b	+/-
K99 (275-295) of VP7	DDA	2+
Whole Virus Control	FCA	-

Diarrhea Scored 4+ (Severe) to - (None)

^a Freund's Complete Adjuvant

^b Dimethyl Dioctyl Decyl Ammonium Bromide

The embodiments of the invention in which an exclusive property. or privilege is claimed are defined as follows:

1. A fragment of the 41K neutralizing glycoprotein (VP7) of rotavirus, said fragment having a molecular weight of 14,000, and having immunological activity capable of eliciting antibodies which neutralize the virus and block viral attachment to host cells comprising the following amino acid sequence:

Cys-Asn-Pro-Met-Asp-Ile-Thr-Leu-Tyr-Tyr-Tyr-Gln-Gln-Thr-Asp-Glu-Ala-Asn-Lys-Trp-Ile-Ser-Met-Gly-Ser-Ser-Cys-Thr-Val-Lys-Val-Cys-Pro-Leu-Asn-Thr-Gln-Thr-Leu-Gly-Ile-Gly-Cys-Leu-Ile-Thr-Asn-Pro-Asp-Thr-Phe-Glu-Thr-Val-Ala-Thr-Thr-Glu-Lys-Leu-Val-Ile-Thr-Asp-Val-Val-Asp-Gly-Val-Asn-His-Lys-Leu-Asn-Val-Thr-Thr-Ala-Thr-Cys-Thr-Ile-Arg-Asn-Cys-Lys-Lys-Leu-Gly-Pro-Arg-Glu-Asn-Val-Ala-Ile-Ile-Gln-Val-Gly-Gly-Ala-Asn-Val-Leu-Asp-Ile-Thr-Ala-Asp-Pro-Thr-Thr-Ala-Pro-Gln-Thr-Glu-Arg-Met-Met-Arg-Ile-Asn-Trp-Lys-Lys-Trp-Trp-Gln-Val or an antigenic equivalent thereof, such as those shown in Figure 1.

2. A peptide of the neutralizing glycoprotein (VP7) of rotaviruses demonstrating similar immunogenic activity of the entire rotavirus glycoprotein or of the rotavirus itself, said peptide comprising the following amino acid sequence: Tyr-Gln-Gln-Thr-Asp-Glu-Ala-Asn-Lys or an antigenic equivalent thereof.

3. A peptide of the neutralizing glycoprotein (VP7) of rotaviruses demonstrating a substantial portion of the immunogenic activity of the entire rotavirus glycoprotein or of the rotavirus itself, said peptide comprising the following amino acid sequence:
Asp-Glu-Ala-Asn-Lys-Lys-Leu-Gly-Pro-Arg-Glu-Asn-Val-Ala or an antigenic equivalent thereof.

4. A peptide of the neutralizing glycoprotein (VP7) of rotaviruses demonstrating a substantial portion of the immunogenic activity of the entire rotavirus glycoprotein

or of the rotavirus itself, said peptide comprising the following amino acid sequence: Arg-Asn-Cys-Lys-Lys-Leu-Gly-Pro-Arg-Glu-Asn-Val-Ala or an antigenic equivalent thereof.

5. A peptide of the neutralizing glycoprotein (VP7) or rotaviruses demonstrating a substantial portion of the immunogenic activity of the entire rotavirus glycoprotein or of the rotavirus itself, said peptide comprising the following amino acid sequence: Pro-Thr-Thr-Ala-Pro-Gln-Thr-Glu-Arg-Met-Met-Arg-Ile-Asn-Trp-Lys-Lys-Trp-Trp-Gln-Val or an antigenic equivalent thereof.

6. A fragment of the 45K nucleocapsid protein (VP6) of rotaviruses, said fragment having a molecular weight of 6,300 and having immunological activity capable of neutralizing an intact virus, said fragment comprising the following amino acid sequence: Thr-Met-Asn-Gly-Asn-Phe-Gln-Glu-Thr-Gly-Gly-Ile-Gly-Asn-Leu-Pro-Ile-Arg-Asn-Trp-Asn-Phe-Asn-Phe-Gly-Leu-Leu-Gly-Thr-Thr-Leu-Leu-Asn-Leu-Asp-Ala-Asn-Tyr-Val-Glu-Thr-Ala-Arg-Asn-Thr-Ile-Asp-Tyr-Phe-Val-Asp-Phe-Val-Asp-Asn-Val-Cys-Met or an antigenic equivalent thereof.

7. A peptide of the nucleocapsid protein (VP6) of rotaviruses said peptide having the immunological activity to simulate antibodies capable of neutralizing the intact virus, said peptide comprising the following amino acid sequence: Thr-Met-Asn-Gly-Asn-Glu-Phe-Gln-Thr-Gly-Gly-Ile-Gly-Asn-Leu-Pro-Ile-Arg-Asn-Trp-Asn or an antigenic equivalent thereof.

8. A fragment corresponding to the trypsin-cleavage site of the 84K neutralizing glycoprotein (VP3) of rotaviruses, said peptide having immunological activity capable of eliciting antibodies which neutralize the virus, and also having the ability to bind and be cleaved by trypsin, to bind to the nucleocapsid protein (VP6) of

bovine rotavirus, and to reduce the rate of virus replication, comprising the following amino acid sequence:

Asn-Ile-Ala-Pro-Ala-Ser-Ile-Val-Ser-Arg-Asn-Ile-Val-Tyr-Thr-Arg-Ala-Gln-Pro-Asn-Gln-Asp-Ile-Ala or an antigenic equivalent thereof.

9. The peptide which is a fragment of the intact VP7 of rotaviruses, said peptide having a molecular weight of 14,000 and having an amino acid sequence of 165 to 295 of the intact VP7, or an antigenic equivalent thereof.

10. The antigenic peptide of the VP7 of rotaviruses, said peptide having an amino acid sequence of 175-1836 of the intact VP7, or an antigenic equivalent thereof.

11. The antigenic peptide of the VP7 of rotaviruses, said peptide having an amino acid sequence of (179-183)-(251-259) of the intact VP7, or an antigenic equivalent thereof.

12. The antigenic peptide of the VP7 of rotaviruses, said peptide having an amino acid sequence of 247-2596 of the intact VP7, or an antigenic equivalent thereof.

13. The antigenic peptide of the VP7 of rotaviruses, said peptide having an amino acid sequence of 275-295 of the intact VP7, or an antigenic equivalent thereof.

14. The 6.3K peptide fragment of the nucleocapsid protein VP6 of rotaviruses, said fragment having an amino acid sequence of 40-976 of the intact VP6, or an antigenic equivalent thereof.

15. The antigenic peptide of the nucleocapsid protein VP6 of rotaviruses, said peptide having an amino acid sequence of 40-606 of the intact VP6, or an antigenic equivalent thereof.

16. The peptide of the VP3 of rotavirus, said peptide having an amino acid sequence of 232-256 of the intact VP3, or an antigenic equivalent thereof.

17. A vaccine for producing immunological activity, comprising an effective amount of a peptide or mixture of

said peptides as defined in claims 1-16 capable of eliciting antibodies capable of neutralizing intact rotaviruses.

18. A vaccine as claimed in claim 17 wherein the peptide is combined with a macromolecular carrier.

19. A vaccine as claimed in claim 18 wherein the macromolecular carrier is selected from keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA), ovalbumin (OVA), β -galactosidase, β -penicillinase, multi-poly-DL-alanyl-poly-L-lysine or poly-L-lysine or VP6 rotavirus protein.

20. A vaccine as claimed in claim 18 wherein the macromolecular carrier is keyhole limpet hemocyanin (KLH).

21. A vaccine against various gastrointestinal disorders and diarrhea caused by rotaviruses comprising a suitable macromolecular carrier having attached thereto a peptide corresponding to at least one of an antigenic fragment of the virus protein 3 (VP3), virus protein 6 (VP6) or virus protein 7 (VP7) of rotaviruses and a portion of said VP3, VP6 or VP7 fragment, which antigenic fragments or portion of said fragments are common to a plurality of differing rotavirus strains and are capable of eliciting antibodies capable of neutralizing each of said intact differing rotavirus strains, said VP6 fragment consisting of the peptide fragment 40-97 having an amino acid sequence of Thr-Met-Asn-Gly-Asn-Glu-Phe-Gln-Thr-Gly-Gly-Ile-Gly-Asn-Leu-Pro-Ile-Arg-Asn-Trp-Asn-Phe-Asn-Phe-Gly-Leu-Leu-Gly-Thr-Thr-Leu-Leu-Asn-Leu-Asp-Ala-Asn-Tyr-Val-Glu-Thr-Ala-Arg-Asn-Thr-Ile-Asp-Tyr-Phe-Val-Asp-Phe-Val-Asp-Asn-Val-Cys-Met; said portion of said VP6 fragment having an amino acid sequence of Thr-Met-Asn-Gly-Asn-Glu-Phe-Gln-Thr-Gly-Gly-Ile-Gly-Asn-Leu-Pro-Ile-Arg-Asn-Trp-Asn; and said VP7 fragment consisting of the peptide fragment 165-295 having the amino acid sequence of:

Cys-Asn-Pro-Met-Asp-Ile-Thr-Leu-Tyr-Tyr-Tyr-Gln-Gln-Thr-Asp-Glu-Ala-Asn-Lys-Trp-Ile-Ser-Met-Gly-Ser-Ser-Cys-Thr-Val-Lys-Val-Cys-Pro-Leu-Asn-Thr-Gln-Thr-Leu-Gly-Ile-Gly-Cys-Leu-Ile-Thr-Asn-Pro-Asp-Thr-Phe-Glu-Thr-Val-Ala-Thr-Thr-Glu-Lys-Leu-Val-Ile-Thr-Asp-Val-Val-Asp-Gly-Val-Asn-His-Lys-Leu-Asn-Val-Thr-Thr-Ala-Thr-Cys-Thr-Ile-Arg-Asn-Cys-Lys-Lys-Leu-Gly-Pro-Arg-Glu-Asn-Val-Ala-Ile-Ile-Gln-Val-Gly-Gly-Ala-Asn-Val-Leu-Asp-Ile-Thr-Ala-Asp-Pro-Thr-Thr-Ala-Pro-Gln-Thr-Glu-Arg-Met-Met-Arg-Ile-Asn-Trp-Lys-Lys-Trp-Trp-Gln-Val;

said portion of the said VP7 fragment being selected from a group of peptide units consisting of: Try-Gln-Gln-Thr-Asp-Glu-Ala-Asn-Lys; Asp-Glu-Ala-Asn-Lys-Lys-Leu-Gly-Pro-Arg-Glu-Asn-Val-Ala; Arg-Asn-Cys-Lys-Lys-Leu-Gly-Pro-Arg-Glu-Asn-Val-Ala; and Pro-Thr-Thr-Ala-Pro-Gln-Thr-Glu-Arg-Met-Met-Arg-Ile-Asn-Trp-Lys-Lys-Trp-Trp-Gln-Val; and

said portion of the VP3 protein consisting of the peptide fragment 232-256 having the amino acid sequence of: Asn-Ile-Ala-Pro-Ala-Ser-Ile-Val-Arg-Asn-Ile-Val-Tyr-Thr-Arg-Gln-Pro-Asn-Gln-Asp-Ile-Ala.

22. A vaccine as defined in claim 21 wherein the peptide is covalently linked to keyhole limpet hemocyanin (KLH).

23. A vaccine as defined in claim 21 wherein the macromolecular carrier is selected from keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) ovalbumin (OVA), β -galactosidase, β -penicillinase, multi-poly-DL-alanyl-poly-L-lysine, poly-L-lysine, or VP6 bovine rotavirus protein.

24. A vaccine as claimed in claim 17 in unit dosage form.

25. A vaccine as defined in claim 17 wherein said vaccine includes a suitable adjuvant.

26. A method for producing immunological activity in mammals or fowl comprising administering to said mammal or

fowl an effective quantity of vaccine as claimed in claim 17.

27. A method for producing immunological activity in mammals or fowl comprising administering to said mammal or fowl an effective quantity of vaccine wherein said vaccine is comprised of at least a peptide selected from the VP7 peptides of claim 1, at least a peptide selected from the VP6 peptides of claim 6, and the peptide from the VP3 peptide of claim 8.

28. A method for producing immunological activity in mammals or fowl comprising administering to said mammal or fowl an effective quantity of vaccine wherein said vaccine is comprised of a peptide selected from the VP7 peptides of claim 1 or claim 9.

29. A method for producing immunological activity in mammals or fowl comprising administering to said mammal or fowl an effective quantity of vaccine wherein said vaccine is comprised of a peptide selected from the VP6 peptides of claim 6 or claim 14.

30. A method for producing immunological activity in mammals or fowl comprising administering to said mammal or fowl an effective quantity of vaccine wherein said vaccine is comprised of a peptide selected from the VP3 peptide of claim 8.

31. A method as in claim 26 wherein the vaccine is administered parenterally.

32. A method as in claim 26 wherein the vaccine is administered orally.

33. A vaccine as claimed in claim 17 wherein the dosage is 1 - 100 ug of peptide per kg of body weight.

34. A vaccine for producing immunological activity, comprising an effective amount of virus protein 3, virus protein 6 and virus protein 7 capable of eliciting antibodies capable of neutralizing the intact rotaviruses.

35. A method for treating or preventing rotavirus infection in mammals or fowl comprising administering to

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said mammal or fowl an effective amount of peptide described in claim 8.

36. A method for inhibiting rotavirus infection or replication in vitro comprising the treatment of infecting virus or culture cells with an effective amount of the peptide described in claim 8.

37. A method for linking or binding macromolecules to VP6 of rotavirus by incorporating into said macromolecules peptides of VP3 as described in claim 8.

FIG. 1

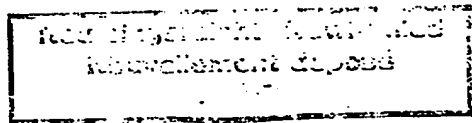
Neu eingereicht / Newly filed
Nouvellement déposé

2/22

5-	GGCTTTTAAAGAACTCTTCAAC	ATG GAT GTC CTA TAC TCT TTG TCA AAG ACT CTT AAA GAC GCT	1465
	ARG ASP LYS ILE VAL GLU GLY THR LEU TYR SER ASN VAL SER ASP LEU ILE GLN GLN PHE	AGA GAC AAA ATT GTC GAA GGC ACA TTG TAT TCT AAC GTG AGT GAT CTA ATT CAA CAA TTT	34125
	ASN GLN MET ILE ILE	THR MET ASN GLY ASN GLU PHE GLN THR GLY GLY ILE GLY ASN LEU AAT CAA ATG ATA ATT ACT ATG AAT GGA AAT GAA TTT CAA ACT GGA GGA ATC GCT AAT TTG	54185
	PRO ILE ARG ASN TRP ASN PHE ASN PHE GLY LEU LEU GLY THR THR LEU LEU ASN LEU ASP	CCA ATT AGA AAC TGG AAT TTT AAT TTC GGG TTA CTT GGA ACA ACT TTG CTG AAC TTA GAC	74245
	ALA ASN TYR VAL GLU THR ALA ARG ASN THR ILE ASP TYR PHE VAL ASP PHE VAL ASP ASN	GCT AAT TAT GTT GAA ACG GCA ASA AAT ACA ATT GAT TAT TTC GTG GAT TTT GTA GAC AAT	94305
	VAL CYS MET ASP	GLU MET VAL ARG GLU SER GLN ARG ASN GLY ILE ALA PRO GLN SER ASP GTA TGC ATG GAT GAG ATG GTT AGA GAA TCA CAA ACG AAC GGA ATT GCA CCT CAA TCA GAC	114365
	SER LEU ARG LYS LEU SER ALA ILE LYS PHE LYS ARG ILE ASN PHE ASP ASN SER SER GLU	TCC CTA AGA AAG CTG TCA GCC ATT AAA TTC AAA AGA ATA AAT TTT GAT AAT TCG TCG GAA	134425
	TYR ILE GLU ASN TRP ASN LEU GLN ASN ARG ARG GLN ARG THR GLY PHE THR PHE HIS LYS	TAC ATA GAA AAC TGG AAT TTG CAA AAT AGA AGA CAG ACG ACA GGT TTC ACT TTT CAT AAA	154485
	PRO ASN ILE PHE PRO TYR SER ALA SER PHE THR LEU ASN ARG SER GLN PRO ALA HIS ASP	CCA AAC ATT TTT CCT TAT TCA GCA TCA TTT ACA CTA AAT AGA TCA CAA CCC GCT CAT GAT	174545
	ASN LEU MET GLY THR MET TRP LEU ASN ALA GLY SER GLU ILE GLN VAL ALA GLY PHE ASP	AAT TTG ATG GGC ACA TRP TGG TTA AAC GCA GGA TCG GAA ATT CAA GTC GCT GGA TTT GAC	194605
	TYR SER CYS ALA ILE ASN ALA ASN ILE GLN GLN PHE GLU HIS ILE VAL PRO LEU	TAC TCA TGT GCT ATT AAC GCA CCA GCC AAT ATA CAA CAA TTT GAG CAT ATT GTG CCA CTC	214665
	ARG ARG VAL LEU THR THR ALA THR ILE THR LEU LEU PRO ASP ALA GLU ARG PHE SER PHE	CGA AGA GTG TTA ACT ACA GCT ACG ATA ACT CTT CTA CCA GAC GCG GAA ACG TTT AGT TTT	234725
	PRO ARG VAL ILE ASN SER ALA ASP GLY ALA THR THR TRP PHE PHE ASN PRO VAL ILE LEU	CCA AGA GTG ATC AAT TCA GCT GAC GGC GCA ACT ACA TGG TTT TTC AAC CCA GTG ATT CTC	254785
	ARG PRO ASN ASN VAL GLU VAL GLU PHE LEU LEU ASN GLY GLN ILE ILE ASN THR TYR GLN	AGG CCG AAT AAC GTT GAA GTG GAG TTT CTA TTG AAT GGA CAG ATA ATA AAC ACT TAT CAA	274845
	ALA ARG PHE GLY THR ILE VAL ALA ARG ASN PHE ASP THR ILE ARG LEU SER PHE GLN LEU	GCA AGA TTT GGA ACT ATC GTA GCT AGA AAT TTT GAT ACT ATT AGA CTA TCA TTC CAG TTA	294905
	MET ARG PRO PRO ASN MET THR PRO ALA VAL ALA VAL LEU PHE PRO ASN ALA GLN PRO PHE	ATG AGA CCA CCA AAC ATG ACA CCA GCA GTA GCA GTA CTA TTC CCG AAT GCA CAG CCA TTC	314965
	GLU HIS HIS ALA THR VAL GLY LEU THR LEU ARG ILE GLU SER ALA VAL CYS GLU SER VAL	GAA CAT CAT GCA ACA GTG GGA TTG ACA CTT AGA ATT GAG TCT GCA GTT TGT GAG TCT GTA	3341025
	LEU ALA ASP ALA SER GLU THR LEU LEU ALA ASN VAL THR SER VAL ARG GLN GLU TYR ALA	CTC GCC ATT GCA AGT GAA ACT CTA TTA GCA AAT GTA ACA TCC GTT ACG CAA GAG TAC GCA	3541085
	ILE PRO VAL GLY PRO VAL PHE PRO PRO GLY MET ASN TRP THR ASP LEU ILE THR ASN TYR	ATA CCA GTT GGA CCA GTC TTT CCA CCA GGT ATG AAC TGG ACT GAT TTA ATC ACC AAT TAT	3741145
	SER PRO SER ARG GLU ASP ASN LEU GLN ARG VAL PHE THR VAL ALA SER ILE ARG SER MET	TCA CCG TCT ACG GAG GAC AAT TTG CAA CCG GTA TTT ACA GTG GCT TCC ATT AGA ACG ATG	3941205
	LEU ILE LYS	CTC ATT AAA TGA GGACCAAGCTAACAACTTGGTATCCAACCTTGGTGAATATGATAGCTATATCAAGCTGTTTGA	3971280
	CTCTGTAAGTAAGGATGCGTATACGCATTGCTACACTGAGTAAATCACTCTGATGATAGTGAGAGGATGAGCC-3'		1357

NUCLEOTIDE SEQUENCE OF CLONED COPY OF SA11 GENE 6. THE (+) SENSE STRAND (corresponding to the mRNA) IS SHOWN. THE PREDICTED AMINO ACID SEQUENCE OF THE PROTEIN PRODUCT IS SHOWN AND THE TERMINATION SITES ARE UNDERLINED.

FIG. 2



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C-406 (bovine) SA-II (ajman)	MASLIYRQLL LIYRQLL	TNSYTVLSD TNSYTVLSD	EIQEIGSTKT EIQEIGSTKT	QNVTVNPGPF QNVTVNPGPF	AQTVASVNW AQTVASVNW	GPGETNDSTT GPGETNDSTT	ALMINO ACID	THREE LETTER SYMBOL	ONE LETTER SYMBOL
61	VEPVLGDPYQ	PITFNPVSY	WMLLAPTHAG	WVGGTNTN	RWLATILIP	NVOOVERTYT	ALANINE	Ala	A
61	VEPVLGDPYQ	PITFNPVSY	WMLLAPTHAG	WVGGTNTN	RWLATILIP	NVOOVERTYT	ARGinine	Arg	R
121	LFQGVQVTV	SNDSTKWK	VLSKOTDGG	NYSHGRLLS	TPKLYGVNKH	GGKIYTYNGE	ASPARAGINE	Asp	N
121	LFQGVQVTV	SNDSTKWK	VLSKOTDGG	NYSHGRLLS	TPKLYGVNKH	GGKIYTYNGE	ASPARTIC ACID	Asp	D
181	TPNATGGYS	TTFDTVMNT	AYCQFYIPL	AGEAKCTEYI	NNGLPIONT	RNIVPVSIVS	ASN AND/OR ASP	Asn	S
181	TPNATGGYS	TTFDTVMNT	AYCQFYIPL	AGEAKCTEYI	NNGLPIONT	RNIVPVSIVS	GLUTAMINE	Gln	C
241	RNIIVYTRAP	NODIIVSKTS	LWKEMQYNRD	IVIRFKFANS	IKSGGLGYK	WSEVSFKPAN	GLUTAMIC ACID	Glu	E
241	RNIIVYTRAP	NODIIVSKTS	LWKEMQYNRD	IVIRFKFANS	IKSGGLGYK	WSEVSFKPAN	GLN AND/OR GLU	Gln	Z
301	YQYTYTROGE	EVTANTCSV	NGINDFNNG	GSLLPTDFVLS	KYEVIKENSF	VYIDYWDSD	HISTIDINE	His	H
301	YQYTYTROGE	EVTANTCSV	NGINDFNNG	GSLLPTDFVLS	KYEVIKENSF	VYIDYWDSD	ISOLEUCINE	Ile	I
361	AFRNWYVRS	LAADLNSVMC	TGGDYSEALP	VGNYPVNTGG	AVSLHSAGVT	LSTQFTDFVS	LEUCINE	Leu	L
361	AFRNWYVRS	LAADLNSVMC	TGGDYSEALP	VGNYPVNTGG	AVSLHSAGVT	LSTQFTDFVS	LYSINE	Lys	K
421	LNSLRFERL	SVEEPFSIL	RTRVSGLYGL	PAKPNNSQE	YYEIAGRFSL	ISLVPSNDDY	METHIONINE	Met	M
421	LNSLRFERL	SVEEPFSIL	RTRVSGLYGL	PAKPNNSQE	YYEIAGRFSL	ISLVPSNDDY	PHENYLALANINE	Phe	F
481	QTPINSVTV	RODLERLGE	LRDEFNNLSQ	QIAMSQILDL	ALLPLDMFSM	FSGIKSTIDA	PROLINE	Pro	P
481	QTPINSVTV	RODLERLGE	LRDEFNNLSQ	QIAMSQILDL	ALLPLDMFSM	FSGIKSTIDA	SERINE	Ser	S
541	AKSMATNMK	RFKKSSLANS	VSTLTDSLSD	AASSISRSAS	VRSVSSTASA	WTEVSNITSD	THREONINE	Thr	T
541	AKSMATNMK	RFKKSSLANS	VSTLTDSLSD	AASSISRSAS	VRSVSSTASA	WTEVSNITSD	TRYPTOPHAN	Trp	W
601	INVTSSIST	QTSTISRRLR	LKENATOTDG	MNFDDISAAV	LTKIDKSTQ	LNTNLTPEIV	TYROSINE	Tyr	Y
601	INVTSSIST	QTSTISRRLR	LKENATOTDG	MNFDDISAAV	LTKIDKSTQ	LNTNLTPEIV	VALINE	Val	V
661	TEASEKFIPI	RAYRVIKODE	VLEASIDGKY	FAYKVETFE	REFSNYKFA	LVTDSPIVSA			
661	TEASEKFIPI	RAYRVIKODE	VLEASIDGKY	FAYKVETFE	REFSNYKFA	LVTDSPIVSA			
721	IDFKTLNL	NONGISROQ	ALNLLRSDPR	VLREFINQDN	PIIRNRIESL	IMCRL			
721	IDFKTLNL	NONGISROQ	ALNLLRSDPR	VLREFINQDN	PIIRNRIESL	IMCRL			

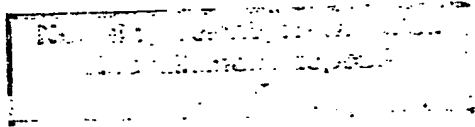
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FIG. 3

AMINO ACID SEQUENCE OF ROTAVIRUS VP3 PROTEIN.

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A B C

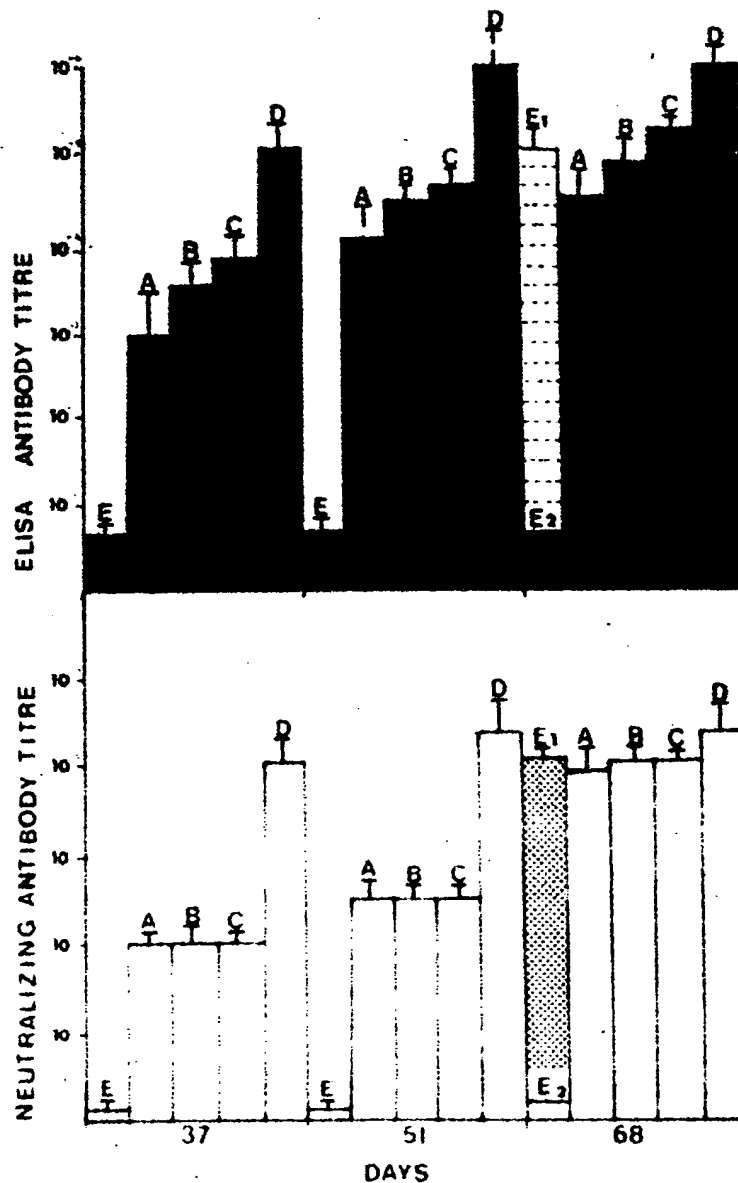


Isolation of BRV 14k peptide fragment. Lanes A and B illustrate the digestion pattern of the BRV glycoprotein produced with 65 ug of papain/cm of gel and 130 ug of papain/cm of gel, respectively. Lane C illustrates the purified 14k peptide fragment. Lanes represent immunoblot-ELISA reactions with monoclonal antibodies from hybridoma 11D12-6. Lane D illustrates the purified 14k peptide fragment in a silver stained polyacrylamide gel.

FIG.4

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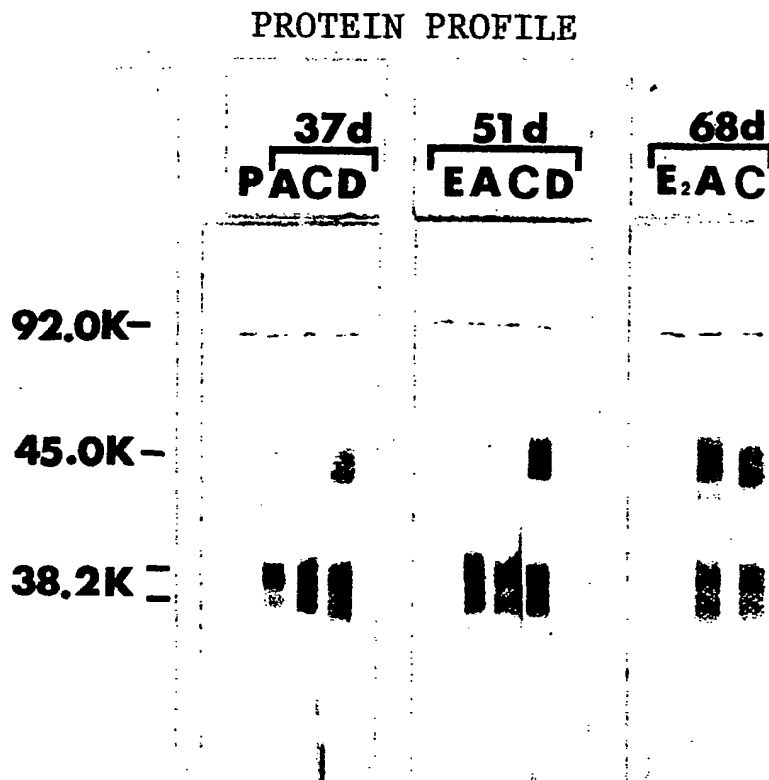
Antibody titers to various preparations of the 14k polypeptide at three different times during the immunization schedule. The upper panel shows total antibody titers as determined by ELISA using double-shelled rotavirus as the antigen. The lower panel shows neutralizing antibody titers as determined by plaque reduction assays. Group A, 14k unconjugated polypeptide; Group B, 14k BSA-conjugated polypeptide; Group C, purified VP7; Group D, infectious bovine rotavirus (BRV); group E₁, animals given infectious BRV at 61 days; group E₂, animals given for each group is described in more detail in Table 2.

FIG. 5

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Nouvellement déposé



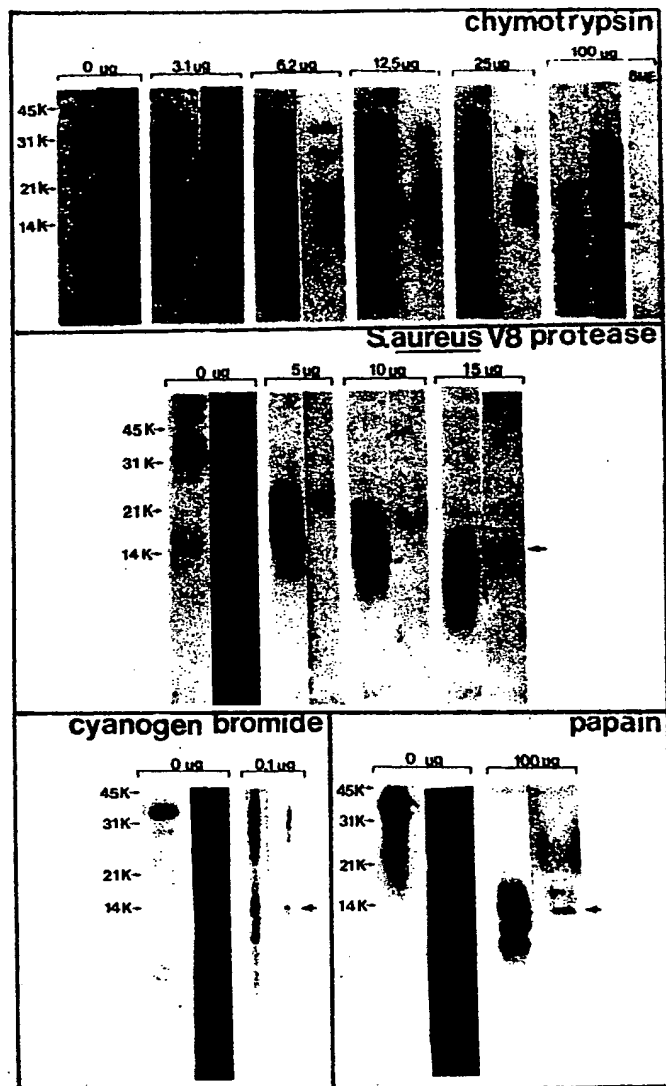
Immuno-blot ELISA reaction of mouse sera with the C486 bovine rotavirus (BRV) protein profile. Lane P, prebleed; A, group given 14k unconjugated polypeptide; C, group given BRV VP7 (38.2k glycoprotein); D, group given infectious virus; E and E₂, groups given saline and Freund's conjugate adjuvant, respectively. The immunization schedule for each group is described in more detail in Table 2.

FIG. 6

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Nouvellement déposé

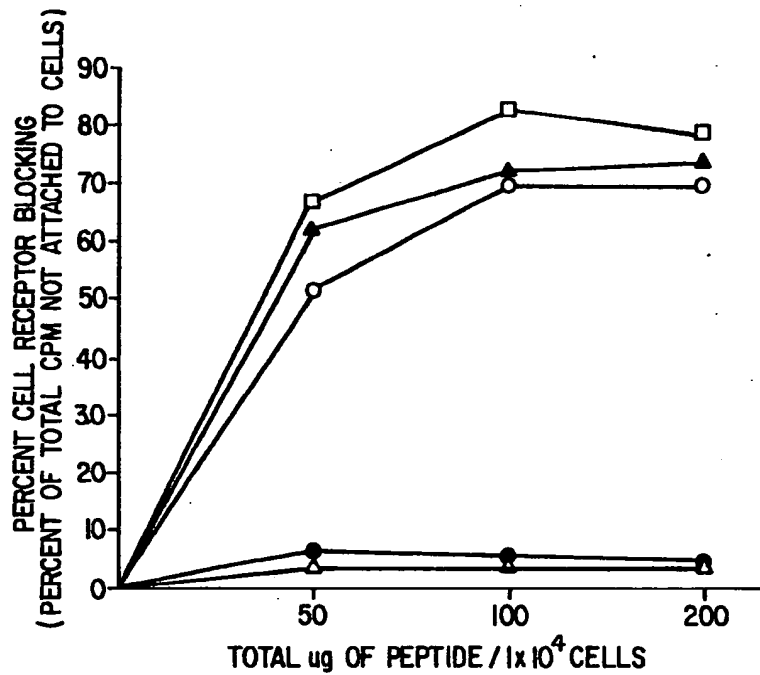


Cleavage patterns of VP7 by Staph aureus V8 protease, papain, chymotrypsin, cyanogen bromide to locate 14k.

FIG. 7

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Nouvellement déposé

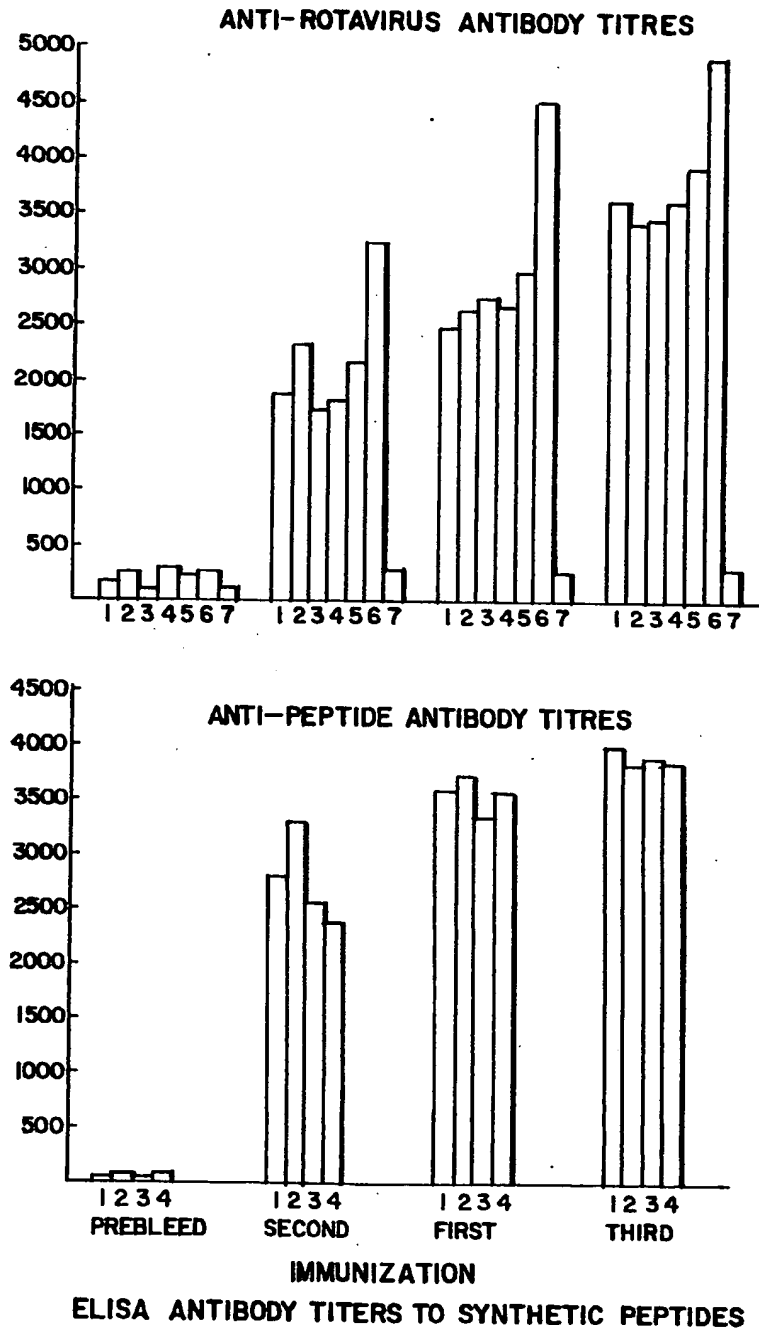


ABILITY OF SYNTHETIC PEPTIDES OF VP7 TO BLOCK VIRUS ATTACHMENT TO CELLS
Various amounts of synthetic peptide were adsorbed to 5×10^4 cells after which time radiolabelled virus was adsorbed to these cells 100% adsorption is equal to 3×10^5 cpm
● p174-183; Δ p178-181; ○ p247-259; □ p275-295; ▲ equal amounts of p247-259 and p275-295.

FIG. 8

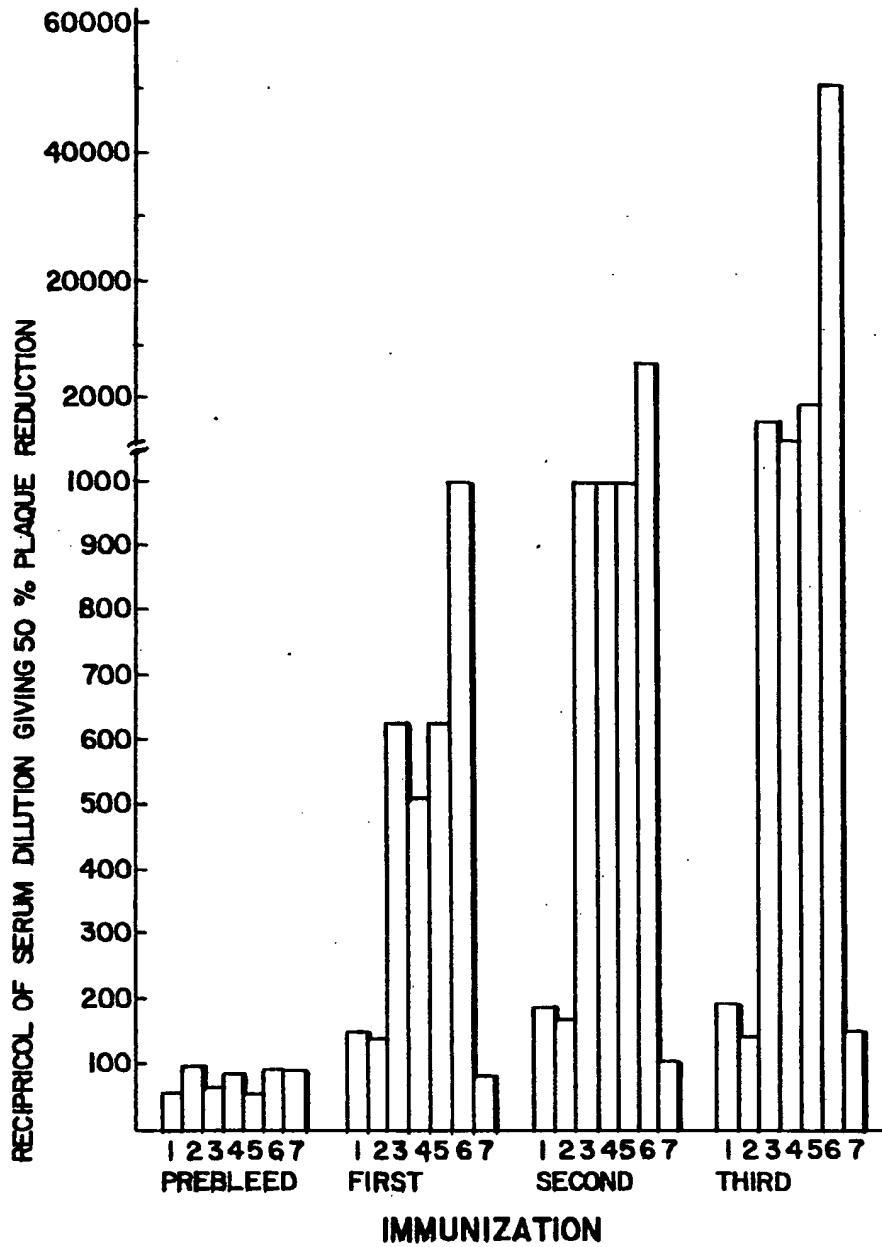
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**FIG.9**

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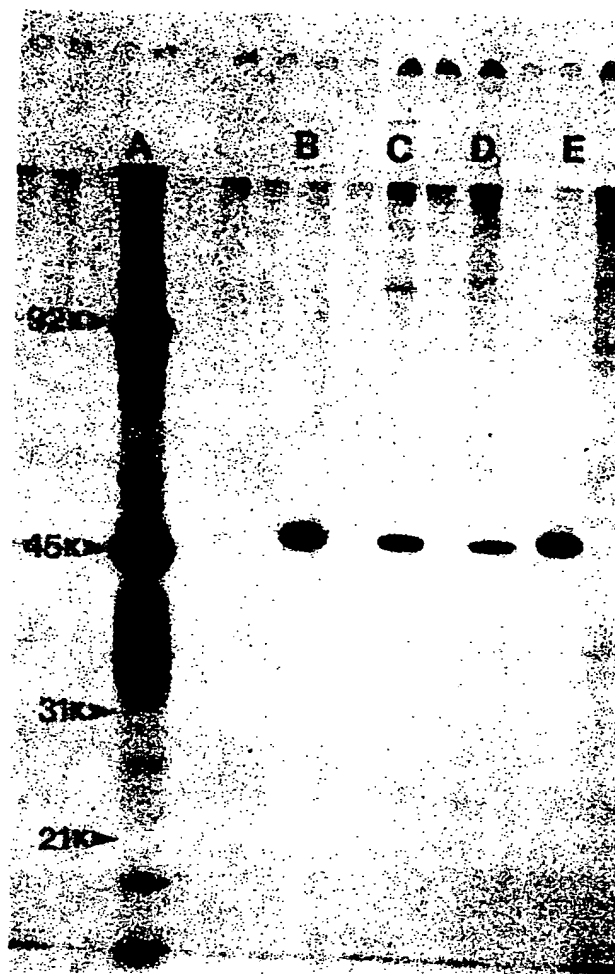
Neu eingereicht / Newly filed
Nouvellement déposé



VIRUS NEUTRALIZING ANTIBODY TITRES INDUCED
IN MICE BY SYNTHETIC PEPTIDES.

FIG. 10

Neu eingereicht / Newly filed
Nouvellement déposé



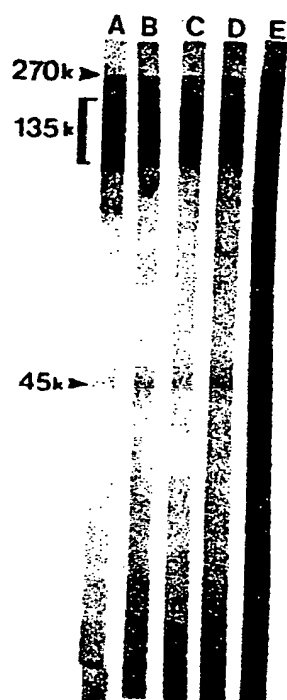
Immunoprecipitation of [35 S] methionine-labelled, bovine rotavirus-infected cell lysate by monoclonal 1D7 (lane B); 1B4 (lane C); 1B9 (lane D) and 1D10 (lane E). Lane A is the reaction with rabbit hyperimmune anti-bovine rotavirus. The position of the molecular weight markers are demonstrated on the left hand side.

FIG. II

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Reaction of monoclonal antibodies and monospecific polyclonal antisera with bovine rotavirus polypeptides transferred to nitrocellulose. Lane A, monoclonal antibody 1D7; lane B, 1B4; lane C, 1B9; lane D, 1D10 and lane E, anti-nucleocapsid monospecific antisera. The molecular weights of the reaction proteins are given on the left hand side.

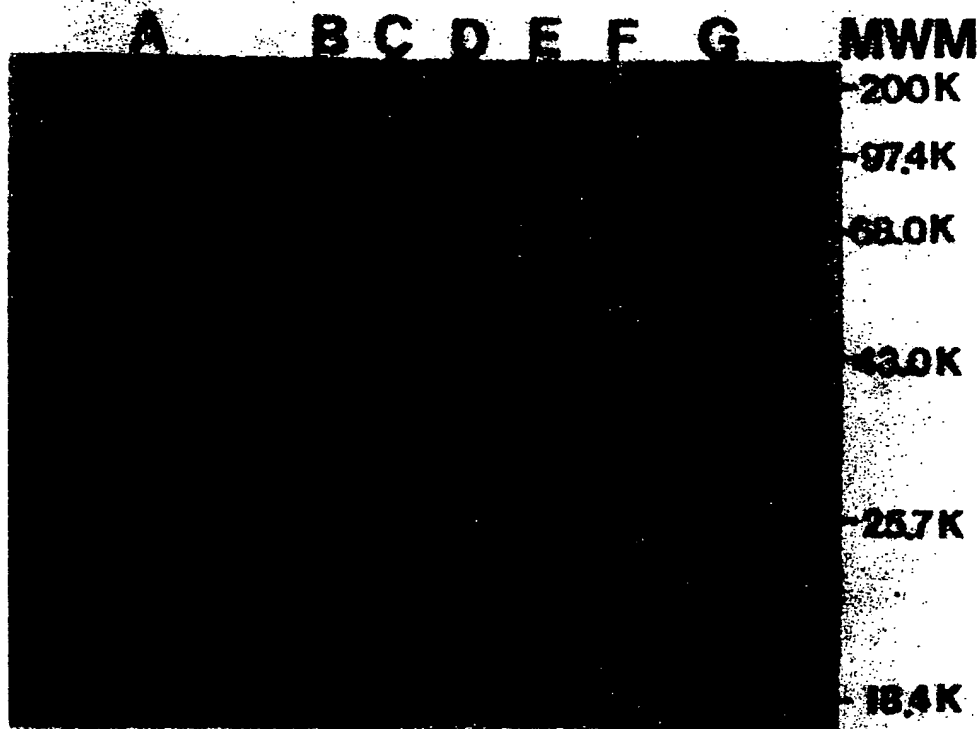
FIG. 12

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Nouvellement déposé

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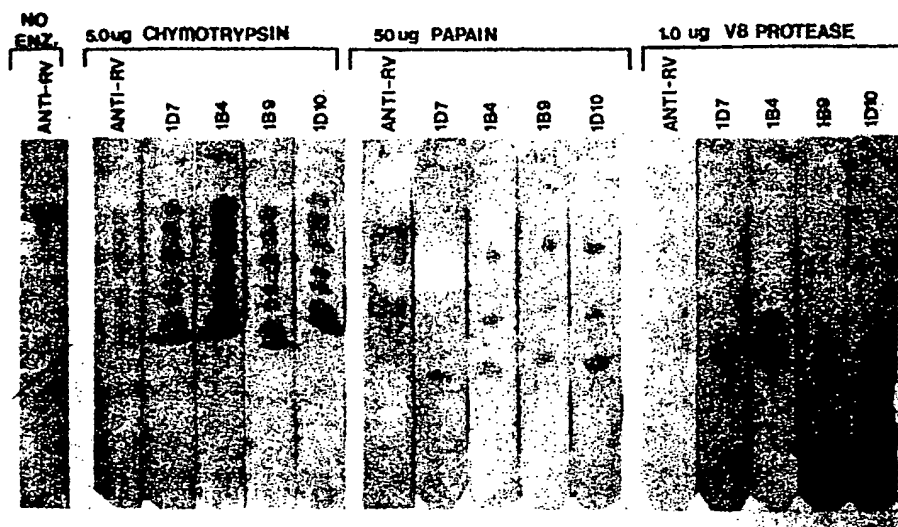


Immuno-blot ELISA reaction of bovine, simian, pig and human rotavirus VP6 nucleocapsid proteins with monoclonal antibody 1B4. Lane A, bovine isolate C486; Lane B, porcine isolate OSU; Lane C, bovine isolate NCDV; Lane D, human isolate Wa; Lane E, bovine isolate UK; Lane F, human isolate ST4; Lane G, simian isolate SA11. Human isolates Wa and ST4 belong to subgroup 2, while all other isolates belong to subgroup 1. Molecular weights are indicated on the right hand side and ascites control is designated.

FIG. 13

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Nouvellement déposé



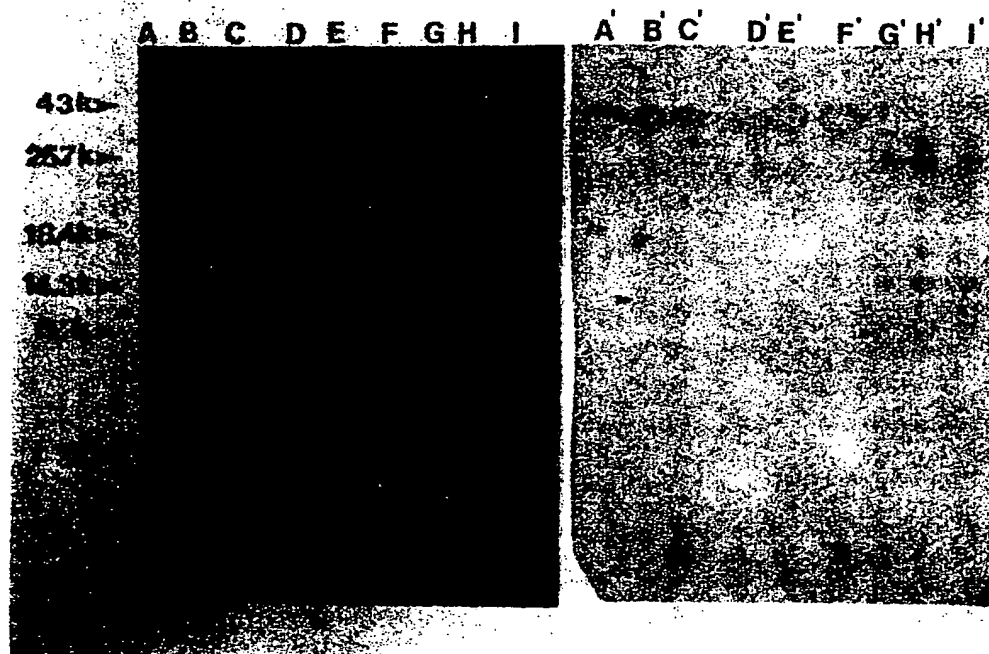
Reaction of bovine rotavirus VP6 nucleocapsid protein digests with monoclonal antibodies 1D7, 1B4, 1B9 and 1D10 and with monospecific antiserum. The enzyme and quantity used per digest are indicated on the figure.

FIG.14

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Nouvellement déposé



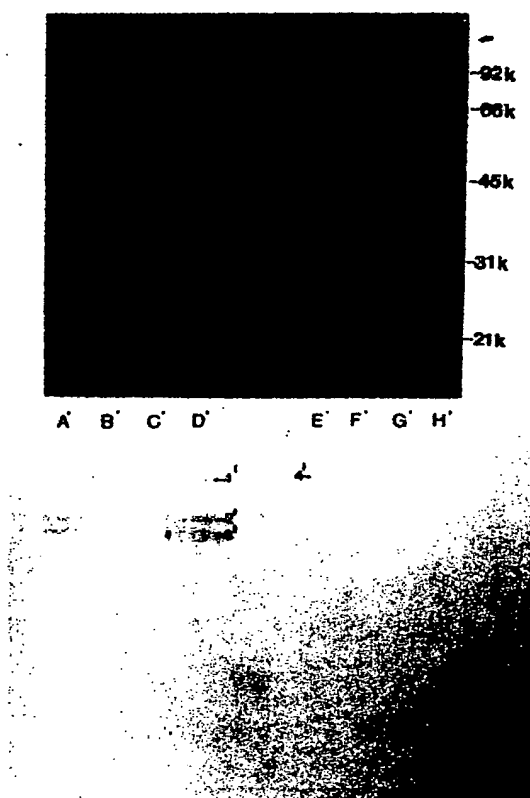
Partial carboxypeptidase digest and cyanogen bromide chemical cleavage of the VP6 nucleocapsid protein and immunoblot ELISA reaction with monoclonal antibody 1B9. Lanes A-I represent the autoradiogram of the digests; Lanes A' -I' represent the corresponding immunoblot ELISA reaction using monoclonal antibody 1B9. Lanes A, A', 100 ug carboxypeptidase; B, B', 50 ug carboxypeptidase; C, C', 25 ug carboxypeptidase; D, D', 2.5 ug carboxypeptidase; E, E', 0.25 ug carboxypeptidase; F, F', undigested nucleocapsid; G, G', 2h digestion with 50 ug cyanogen bromide; H, H', 1 h digestion with 50 ug cyanogen bromide; I, I', 30 min digestion with 50 ug cyanogen bromide. The positions of molecular weight standards are indicated on the left hand side of the figure.

FIG. 15

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Nouvellement déposé



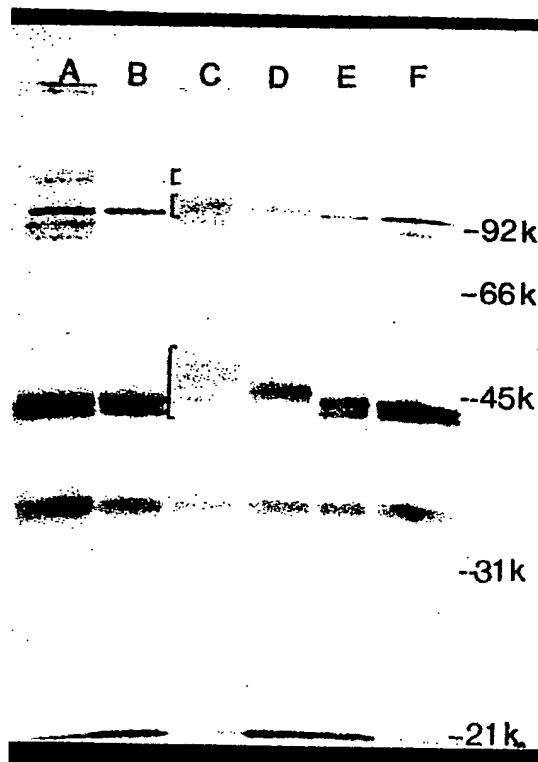
The reaction of antiserum to the synthetic peptide 232-256 of VP3 with the rotavirus protein profile. The upper panel (Lane A-H) represents the autoradiograph and the lower panel (Lanes A'-H') represents the immunoblot-ELISA reaction of the protein profile shown in the upper panel with anti-synthetic peptide serum. Lanes A-D represent protein profiles generated in the presence of Laemmli sample buffer containing BME and boiled, and Lanes E-H represent protein profiles generated in the presence of Laemmli sample buffer without BME but boiled. Lanes A, A' and E and E' represent a mixture of 100 ug of synthetic peptide, 2.0 ug double-shelled rotavirus, and .96 ug trypsin. Lanes B and B' and F and F' represent a mixture of 25 ug of synthetic peptide, 2.0 ug of double-shelled rotavirus and .96 ug trypsin. Lanes C and C'; D and D' and G and G' represent 2.0 ug of double-shelled rotavirus and .96 ug trypsin. Lanes H and H' represent double-shelled virus. The numbers indicate position of various proteins referred to in the text. Molecular weight markers are indicated on the right hand side.

FIG.16

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Nouvellement déposé



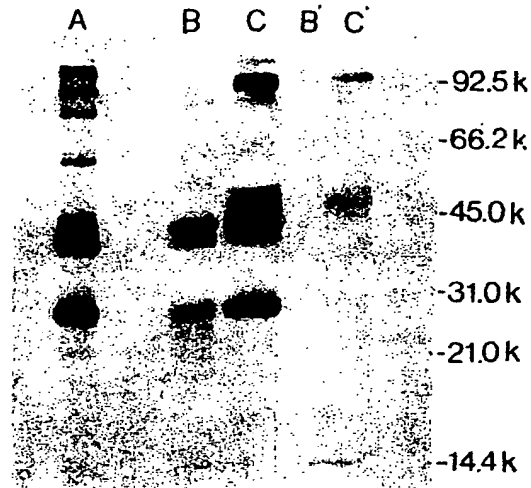
Reactivity of synthetic peptide with the VP3 nucleocapsid protein of bovine rotavirus. Lane A represents the protein profile after reacting 2.0 ug of double-shelled virus with 25 ug of synthetic peptide, Lane B represents the same profile as in A after treatment with .96 ug of trypsin. Lane C represents the protein profile after reacting 2.0 ug of double-shelled virus with 100 ug of synthetic peptide and Lane D represents the same profile as in C after treatment with 0.96 ug of trypsin. Lane E represents the protein profile of 2.0 ug of virus treated with 0.96 ug of trypsin and Lane F represents the protein profile of 2.0 ug of untreated double-shelled virus. The position of molecular weight standards are indicated on the right hand side. The brackets ([]) in Lane C indicate ladders formed in the 45K, 90K and 135K region.

FIG.17

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Nouvellement déposé



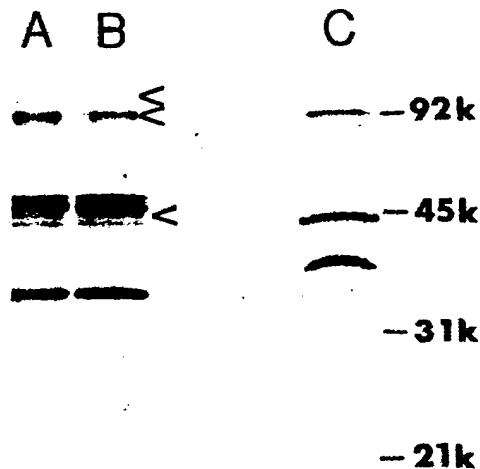
Reactivity of antisera against the 232-256 VP3 synthetic peptide with the ladder complex on VP6 nucleocapsid monomers and dimers. Lane A represents the virus protein profile. Lane B represents the virus protein profile after complexing with 100 ug of the synthetic peptide and then treatment with .96 ug of trypsin; Lane B' represents the virus profile in Lane B electroblotted and reacted with anti-synthetic peptide antibodies. Lane C represents the virus protein profile after complexing with 100 ug of the synthetic peptide; Lane C' represents the virus profile in Lane C electroblotted and reacted with anti-synthetic peptide antibodies. The right hand side illustrated the location of molecular weight markers.

FIG.18

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Nouvellement déposé



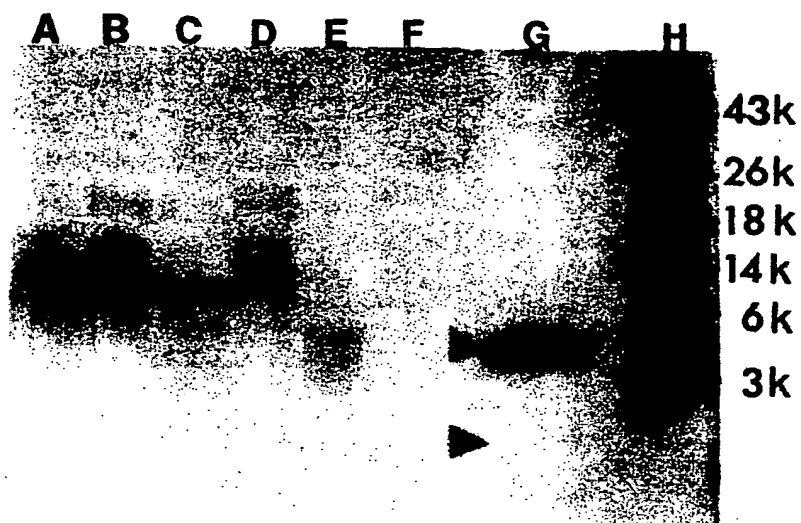
Investigation of sample buffer conditions necessary to maintain the 232-256 VP3 synthetic peptide-VP6 complex. Two micrograms of radiolabelled double-shelled rotavirus was reacted with 100 ug synthetic peptide for 30 min at 37° C. Prior to electrophoresis the sample was aliquoted and treated with urea sample buffer, Lane A; Laemmli sample buffer without BME, Lane B; and Laemmli sample buffer containing BME and boiled, Lane C. The arrowheads indicate the position of the 45K, 90K, 135 ladders. The position of the molecular weight standards are located on the right hand side.

FIG.19

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Nouvellement déposé



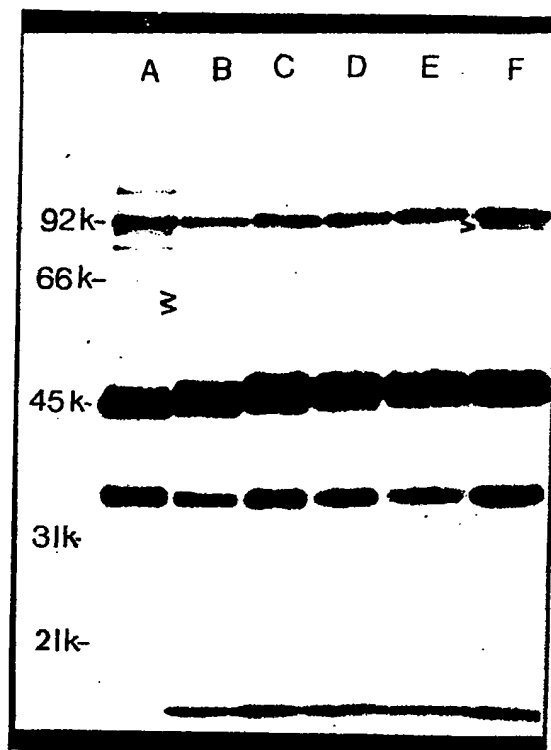
Trypsin cleavage of the synthetic peptide. Lanes B, D, and F represent trypsin at 19.2 ug, 9.6 ug and .96 ug respectively. Lane A, C and E represent the reaction of 100 ug of synthetic peptide with 19.2 ug, 9.6 ug and .96 ug of trypsin, respectively. Lane G represents 100 ug of the peptide with the arrows indicating the position of the monomer and dimer. Lane H indicates the position of molecular weight markers.

FIG.20

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Nouvellement déposé



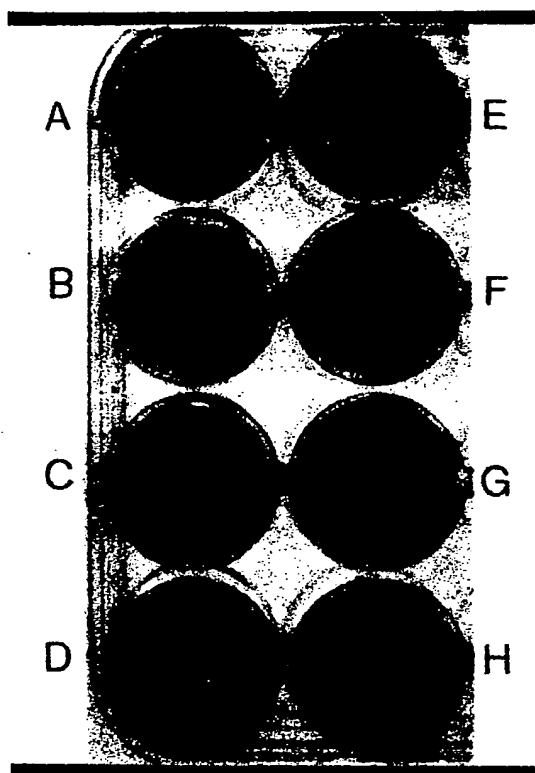
Competition of 232-256 VP3 synthetic peptide with the intact 84,000 VP3 for trypsin. Lane A represents the protein profile of double-shelled rotavirus. Lane C-F represents the viral protein profile after incubation of virus for 30 min at 37° C with .0097 ug of trypsin and increasing amounts of synthetic peptide. Lane B represents no synthetic peptide, Lane C has 25 ug; Lane D, 50 ug; Lane E, 75 ug; and Lane F, 200 ug of synthetic peptide. The positioned molecular weight markers are indicated on the left hand side. The arrowheads at Lane B denote the position of the doublet observed at 60,000 and the arrowhead at Lane F denotes the position of the 84,000 protein.

FIG. 21

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Nouvellement déposé



Effect of increasing amounts of synthetic peptide on the infectivity of rotavirus. Well A represents MA-104 cell control, well B represents the virus control approximately 100 PFU. Wells C-H represent duplicate samples of 100 PFU adsorbed to MA-104 cell monolayers in the presence of 100 ug, 200 ug and 300 ug of synthetic peptide, respectively.

FIG.22